

Ribonucleotide Reductase as a Target to Control Apicomplexan Diseases

James B. Munro and Joana C. Silva*

Department of Microbiology and Immunology and Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Abstract

Malaria is caused by species in the apicomplexan genus *Plasmodium*, which infect hundreds of millions of people each year and kill close to one million. While malaria is the most notorious of the apicomplexan-caused diseases, other members of the eukaryotic phylum Apicomplexa are responsible for additional, albeit less well-known, diseases in humans, economically important livestock, and a variety of other vertebrates. Diseases such as babesiosis (hemolytic anemia), theileriosis and East Coast Fever, cryptosporidiosis, and toxoplasmosis are caused by the apicomplexans *Babesia*, *Theileria*, *Cryptosporidium* and *Toxoplasma*, respectively. In addition to the loss of human life, these diseases are responsible for losses of billions of dollars annually. Hence, the research into new drug targets remains a high priority. Ribonucleotide reductase (RNR) is an essential enzyme found in all domains of life. It is the only means by which *de novo* synthesis of deoxyribonucleotides occurs, without which DNA replication and repair cannot proceed. RNR has long been the target of antiviral, antibacterial and anti-cancer therapeutics. Herein, we review the chemotherapeutic methods used to inhibit RNR, with particular emphasis on the role of RNR inhibition in Apicomplexa, and in light of the novel RNR R2_e2 subunit recently identified in apicomplexan parasites.

Introduction

The Apicomplexa are a group of single-celled, eukaryotic organisms that, together with the ciliates and dinoflagellates, form the major lineages in the Alveolates. All Apicomplexa, save the predatory flagellates Colpodellida, are pathogenetic, obligate, intracellular parasites (Adl et al., 2005; Morrison, 2009). They are characterized by the presence of an apical complex, a structure involved in host-cell invasion, which is located at the anterior end of the cell. Most apicomplexans also possess a specialized organelle called the apicoplast, a secondary endosymbiotic plastid believed to be of red algal origin (Blanchard and Hicks, 1999; Fast et al., 2001; Janouškovec et al., 2010). Within the apicoplast occur processes essential for the parasite's survival, such as heme and lipid biosynthesis. Another defining characteristic of apicomplexans is their inability to synthesize purine rings *de novo* and hence their need to salvage exogenous purines via a variety of different pathways (Booden and Hull, 1973; Chaudhary et al., 2004; de Koning et al., 2005; Cassera et al., 2008; Madrid et al., 2008). These organisms have complex (indirect) life cycles, and they often exploit multiple

hosts/vectors and transition between life cycle stages is dependent upon a diverse array of environmental cues.

The biological characteristics that differentiate apicomplexans from their vertebrate hosts have often been considered optimal targets of new therapeutics to control these eukaryotic pathogens. Alternatively, essential and strongly conserved proteins can be targeted, provided that they differ from their vertebrate homologs in such a way that minimizes potential cross-reaction and toxicity.

The enzyme ribonucleotide reductase (RNR) is one such example. RNR utilizes free radical chemistry to catalyze the reduction of ribonucleotides to deoxyribonucleotides (Thelander and Reichard, 1979; Reichard, 1988). It provides the only *de novo* means of generating the essential building blocks for DNA replication and repair across all domains of life and, as such, it is the rate-limiting step in DNA synthesis (Jordan and Reichard, 1998; Lundin et al., 2009). Additionally, RNR is critical for maintaining a balanced pool of DNA precursors during chromosome replication (Herrick and Sclavi, 2007). An unbalanced deoxyribonucleotide triphosphate pool may lead to an increase in mutation and disease (Lin and Elford, 1980; Reichard, 1988; Chabes et al., 2003; Wheeler et al., 2005; Gon et al., 2006; Mathews, 2006; Kumar et al., 2010).

Here we review the chemotherapeutic methods used to inhibit the essential enzyme RNR, with particular emphasis on the novel RNR R2_e2 subunit recently identified in apicomplexan parasites (Munro et al., submitted) and on the malaria-causing genus *Plasmodium*. The R2_e2 subunit is unique to the Apicomplexa and as such, it can potentially be used to specifically target apicomplexan pathogens.

Apicomplexan parasites are responsible for devastating infectious diseases

The phylum Apicomplexa consists of more than 4,000 described species (Levine, 1988), many of which are of medical, agricultural, and economic importance and whose adverse impact on human society cannot be overstated. Among the most notorious are *Plasmodium*, *Babesia*, *Theileria*, *Cryptosporidium*, and *Toxoplasma* the causative agents of malaria, babesiosis, theileriosis and East Coast fever, cryptosporidiosis, and toxoplasmosis, respectively. They are responsible for causing millions of human deaths and billions of dollars in productivity and material losses each year (Sachs and Malaney, 2002; Corso et al., 2003; Rowe et al., 2006; Spielman, 2009). Currently, five species of *Plasmodium* are known to cause malaria in humans, *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax* (Rougemont et al., 2004; Singh et al., 2004; Cox-Singh et al., 2008), of which *P. falciparum* is the most deadly and *P. vivax* the most geographically widespread. The life cycle of *Plasmodium* alternates between a vertebrate host and mosquito vector and involves four major developmental stages in the vertebrate host: sporozoites, merozoites, trophozoites, and gametocytes (Bledsoe, 2005; Brown and Catteruccia, 2006).

*Corresponding author: jcsilva@som.umaryland.edu

Prioritization of apicomplexan drug targets

There is currently no fully efficacious vaccine on the market against any apicomplexan species and drug treatment is the method of choice in the management of apicomplexan diseases. Modern approaches to drug design, made possible with the advent of genome sequencing, emphasize defining and targeting metabolic and molecular differences between host and parasite to avoid host side effects (Croft, 1997). This may be achieved with the selective targeting of parasite-specific enzymes or by targeting those which are highly divergent or have distinct binding sites and are thus sufficiently different to be selectively targeted (Coombs, 1999; Cerqueira et al., 2007). Also important is that the protein target be essential to the growth, reproduction, or survival of the parasite. Finally, knowledge of the gene expression pattern of potential target proteins is necessary to link drug administration with the critical and relevant stages of the pathogen's life cycle. This is particularly pertinent to organisms characterized by differentially expressed, stage-specific, and often stage-unique gene expression, such as apicomplexans (Coulson et al., 2004). It has been suggested that targeting the intraerythrocytic life stages of *Plasmodium* (the ring forms, trophozoites, schizonts, and merozoites), when clinical symptoms are manifested, is of particular interest (Yeh and Altman, 2006). However, targeting multiple life stages, for example controlling both the liver and blood stages of *Plasmodium*, may be more conducive to the ultimate goal of disease eradication (Alonso et al., 2011).

Current control strategies and challenges

Drug targets for control of apicomplexans have focused on parasite metabolic processes. These include processes within the cytosol, mitochondrion, digestive vacuole, synthesis of macromolecular and metabolic enzymes, and processes involved in membrane synthesis and signaling (Olliaro and Yuthavong, 1999; Padmanaban, 2003; Fidock et al., 2004; El Bissati et al., 2006). Enzymes in the purine salvage pathways, essential to these pathogens, are also potential drug targets (Tracy and Sherman, 1972; Krug et al., 1989; Parker et al., 2000; Gardner et al., 2002; Raman and Balaram, 2004; Striepen et al., 2004; Ting et al., 2005; Downie et al., 2008), as are all proteins and processes related to the apicomplexan-specific organelle, the apicoplast (Foth et al., 2003; Sato and Wilson, 2005; Wiesner et al., 2008; Lizundia et al., 2009). Antibiotics like doxycycline, which specifically target plastid pathways and impair the expression of apicoplast genes, can be used to target apicomplexans (Ralph et al., 2001; Leander and Keeling, 2003; Dahl et al., 2006). Apicoplast drug targets have included the apicoplast's metabolic pathways (e.g. DNA replication, transcription, protein translation, fatty acid biosynthesis, and isoprenoid biosynthesis), or targeting proteins encoded by the host's nuclear genes that are destined for the apicoplast (McFadden and Roos, 1999; Roos et al., 1999; Roos et al., 2002; Ralph et al., 2004; White, 2004; Waller and McFadden, 2005; Dahl and Rosenthal, 2008; Prusty et al., 2010).

The availability of genome sequences from several species and isolates of *Plasmodium* and other Apicomplexa facilitates the identification of novel, potential drugs for the control of apicomplexan parasites (Doolan et al., 2003; Yeh et al., 2004; Carvalho and Ménard, 2005; Winzeler, 2008; Mu et al., 2010). For example, evolutionary patterning has

been proposed as a means to combat the emergence of drug resistance (Durand et al., 2008). Genes with high rates of nonsynonymous changes have been associated with drug resistance in *P. vivax* (Dharia et al., 2010), and may be responsible for vaccine evasion in *P. falciparum* (Takala and Plowe, 2009). In contrast, evolutionary patterning focuses on finding and targeting protein residues that are under strong purifying selection, which will in principle reduce the instances of drug resistance mutations.

The tremendous health burden imposed by malaria has made *Plasmodium* the primary target for many of these approaches. Despite a diversified arsenal of potential tools to combat malarial infection, multiple drug resistance to existing anti-malarial compounds is becoming increasingly common in *Plasmodium* (Greenwood and Mutabingwa, 2002; Anderson, 2009; Bustamante et al., 2009; Takala and Plowe, 2009). A case in point is the antifolate drugs used to treat malaria. Antifolate drugs bind enzymes necessary for folate biosynthesis, thus targeting essential precursors for purine and pyrimidine synthesis. Antifolates have been used against *Plasmodium* with success, but resistance to these drugs has become widespread (Gregson and Plowe, 2005; Mkulama et al., 2008; Sridaran et al., 2010). Currently, the primary treatment for malaria is based on artemisinin, which is administered in combination with other drugs in order to prevent, or delay, the onset of resistance. However, there are clear indications that artemisinin resistance is emerging (Chrubasik and Jacobson, 2010; Dondorp et al., 2010; Enserink, 2010; Fidock, 2010). Therefore, the development of new chemotherapeutic and prophylactic antimalarial drugs and vaccines remains a priority (Greenwood and Mutabingwa, 2002; Anderson, 2009; Bustamante et al., 2009; Takala and Plowe, 2009).

RNR classification and distribution

RNRs are classified into one of three classes, I-III (Jordan et al., 1994; Reichard, 1997; Fontecave, 1998; Nordlund and Reichard, 2006). All three classes use a thiyl radical to remove the ribose OH-group. The distinction between these classes relies on differences in radical generation chemistry and the cofactor needed to produce the organic radical. Class I RNRs are oxygen-dependent, typically require a tyrosyl radical and a diiron center, and are characteristic of eukaryotes and common among bacteria. Class II RNRs are indifferent to oxygen, form a thiyl radical via adenosylcobalamin, and are characteristic of Archaea and bacteria. Class III RNRs are anaerobic, form a glycy radical using an iron-sulfur center in the presence of S-adenosylmethionine and reduced flavodoxin, and are also characteristic of Archaea and bacteria.

Class I RNRs have been further categorized as class Ia, Ib, or Ic. Class Ia is typical in eukaryotes and bacteria, while bacteria and Archaea primarily encode classes Ib and Ic RNRs (Harder, 1993; Sjöberg, 2010). Class Ic RNRs are further categorized as R2c proteins and while the R2-homolog R2lox proteins are described as "R2c-like", they are not believed to form active RNR holoenzymes (Högbom et al., 2004; Andersson and Högbom, 2009). This classification, based on structural and chemical properties, has shortcomings since classes Ia and Ic are not monophyletic clades, i.e., discrete, mutually exclusive groups, which contain a most recent common ancestor and all of its descendants. Instead, class Ia is polyphyletic and Ic

is paraphyletic. This problem was been addressed by Munro et al. (submitted) (Figure 1) who recognized four distinct class I RNR clades, namely the eukaryotic-specific clades R2_e1 and R2_e2, and the clades containing primarily archaeal and bacterial RNRs, namely R2_ab and R2c. The study also revealed that the newly discovered clade R2_e2 is unique to the Apicomplexa (Munro et al., submitted). In fact, the most significant mammalian-infecting Apicomplexa genera, such as *Plasmodium*, *Cryptosporidium*, and *Babesia*, all encode one R2_e2 subunit.

Class Ia holoenzyme regulation and formation

Apicomplexans encode Class Ia RNRs, which are thus the focus of this review. Class Ia RNR enzymes are composed of two distinct subunits, R1 and R2. Subunit R1, the larger of the subunits, contains a catalytic site (substrate binding)

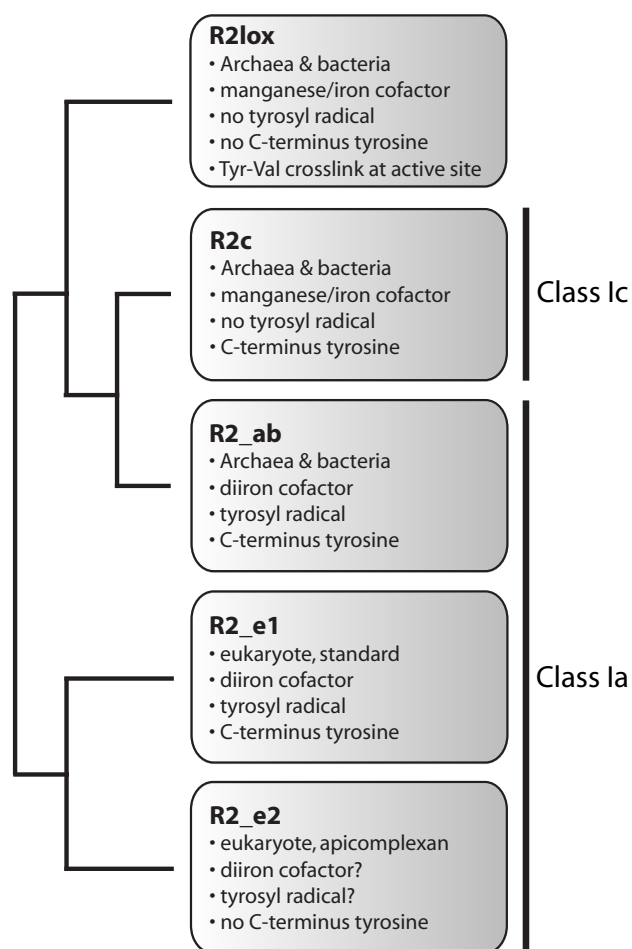


Figure 1. Unrooted phylogenetic relationships between the RNR class Ia and Ic subunits and the R2lox R2 homolog proteins (Munro et al., submitted). Class Ic includes the R2c proteins; however, this classification proved to be paraphyletic as it failed to include the clade of proteins now designated as R2_ab. Former class Ia proved to be polyphyletic, including the novel R2_ab clade, which does not share a recent common ancestor with the monophyletic R2_e1 and R2_e2 clade. Reference to the clades R2c, R2_ab, R2_e1, and R2_e2 now allows for unambiguous reference to these RNR subunits.

and two allosteric effector-binding sites. dATP and ATP bind to the first allosteric site, the A-site, and serve as inhibitor and stimulator, respectively, while binding of dATP, ATP, dGTP and dTTP to the S-site determines substrate-binding preference (Brown and Reichard, 1969; Reichard et al., 2000). Allosteric regulation is accomplished by changes in the conformation of loop 2 which spans both the A- and S-sites, and which is determined by the molecule bound to the A-site (Reichard, 2010). Reviews and additional details can be found in (Eriksson et al., 1979; Reichard et al., 2000; Reichard, 2002; Crona et al., 2010; Logan, 2011).

Subunit R2, the smaller subunit, contains an amino acid residue that harbors the organic radical (Nordlund et al., 1990; Nordlund and Eklund, 1993). A long-range electron-coupled pathway connects the R2 radical to a cysteine in R1 (the thiyl radical) via hydrogen-bonded amino acid residue side chains (Nordlund et al., 1990; Stubbe et al., 2003; Kolberg et al., 2004). Binding of the R2 subunit to the R1 subunit involves the C-terminus residues of the R2 subunit interacting with a hydrophobic cleft in the R1 subunit and it has been suggested that oligomerization of R1 is a prerequisite (Climent et al., 1992; Rova et al., 1999; Uppsten et al., 2006).

The most current model for RNR in eukaryotes suggests a holoenzyme with eight subunits and is of the form $\alpha_6\beta_2$, where alpha stands for the R1 subunit and beta for R2 (Rofougaran et al., 2006). It has been proposed that in the absence of the effectors dATP, ATP, dGTP and dTTP, the R1 subunit is an inactive monomer; however, once dTTP or dGTP are bound to the S-site, an $\alpha_2\beta_2$ heterodimer is formed (Ingemarson and Thelander, 1996). With increasing dATP concentration (which induces enzyme inhibition), R1 monomers form dimers and eventually inactive hexamers (formation of intermediate tetramers remains in question), while in the presence of the enzyme activator ATP, the holoenzyme adopts an $\alpha_6\beta_2$ conformation (Fairman et al., 2011).

Paralogous copies of the R2 subunit

Many eukaryote genomes encode two or more distinct copies of the small R2 subunit (Lundin et al., 2009). For example, humans have the R2 and p53R2 paralogs and *S. cerevisiae* the Y2 and Y4 paralogs. It is clear that these copies have different functional roles, be it *de novo* creation of deoxyribonucleotides, maintenance of the deoxyribonucleotide pool, mitochondrial DNA replication, or DNA damage repair (Elledge and Davis, 1990; Huang and Elledge, 1997; Tanaka et al., 2000; Lin et al., 2004; Bourdon et al., 2007). In such instances, a $\beta\beta'$ configuration is believed to contribute to the active holoenzyme, although $\beta\beta$ and $\beta'\beta'$ configurations have been reported (Wang et al., 1997; Nguyen et al., 1999; Chabes et al., 2000; Ge et al., 2001; Guittet et al., 2001; Voegtli et al., 2001; Perlstein et al., 2005; Ortigosa et al., 2006).

While most eukaryotes encode two R2 genes belonging to the typical eukaryotic clade R2_e1, apicomplexans encode one R2_e1 subunit and one R2_e2 subunit. *Toxoplasma* appears to be an exception, as so far two R2_e1-encoding genes have been identified but no R2_e2 has been found in its genome.

The conservation of functionally important R1 active site cysteines, and electron transfer cysteine and tyrosine residues, as well as the conservation of R2 residues involved

in iron binding, electron transport, free radical transfer, and the formation of the hydrophobic pocket around the radical, implies that both eukaryotic pathogens and their hosts utilize the same free radical chemistry to synthesize deoxyribonucleotides (Hofer et al., 1997; Roshick et al., 2000; Akiyoshi et al., 2002; Shao et al., 2006). As such, it would appear that targeting apicomplexan RNR by chemotherapeutic means might have an adverse effect on the human host. This is not necessarily so.

While prokaryotic and eukaryotic R1 and R2 subunits are highly conserved at, and around, the functionally important residues (Chakrabarti et al., 1993; Sjöberg, 1997; Roshick et al., 2000; Voegtli et al., 2001; Högbom et al., 2004; Högbom, 2010), there is considerable variation in the sequences at both the N- and C-termini (Ingram and Kinnaird, 1999). In particular, the eukaryotic orthodox R2 subunit, R2_e1, presents distinct differences between apicomplexans and mammals, including differences in key functional regions of the R2 protein; perhaps most notable are those differences between C-terminal sequences (Bracchi-Ricard et al., 2005). Furthermore, and more pertinent to this review, is the fact that the apicomplexan-specific R2 subunit, R2_e2, offers additional unique regions for drug-targeted inhibition (Munro et al., submitted). It is these differences between apicomplexan and mammalian host sequences that may best be exploited when designing chemotherapeutic drugs to specifically target the Apicomplexa, making RNR an appealing option as a drug target.

RNR has a long history as chemotherapeutical target

Much research has focused on the relationship between the class Ia R1 and R2 subunits in the context of human cancer. It has been hypothesized that normal or over-expression of R1 results in suppression of malignant cells (Yen, 2003) and Fan et al. (Fan et al., 1997) demonstrated that the R1

subunit had tumor-suppressing activity. Cao et al. (Cao et al., 2003) utilized a recombinant adenovirus that encoded and over-expressed the human R1 gene, which reduced proliferation of human colon adenocarcinoma cells, yet had no effect on normal cells. On the other hand, inhibition of the R2 subunit may have an antineoplastic effect, serving to inhibit and combat the development of cancer cells. Expression of R2 in conjunction with activated oncogenes impacts a cell's malignant potential (Fan et al., 1998; Desai et al., 2005). Overexpression of R2 is linked to increased drug resistance and increased invasive potential in cancer cells (Yen, 2003).

RNR inhibition has been applied to the control of viruses (Gaudreau et al., 1987; Moss et al., 1993; Bianchi et al., 1994; Szekeres et al., 1997; Robins, 1999), bacteria (Yang et al., 1997; Mdluli and Spigelman, 2006; Ericsson et al., 2010; Lou and Zhang, 2010; Torrents and Sjöberg, 2010), and certain cancers (Cory, 1988; Nocentini, 1996; Gwilt and Tracewell, 1998). Because inhibition of RNR ceases, or severely reduces, DNA replication, it has long been considered an ideal target for the control of pathogens. As such, inhibition of RNR to control eukaryotic pathogens has also been suggested (Dormeyer et al., 1997; Ekanem, 2001), in particular those belonging to Apicomplexa (Chakrabarti et al., 1993; Barker et al., 1996; Akiyoshi et al., 2002). In fact, RNR was included in a set of 57 "gold standard" essential enzymes with experimentally documented antimalarial effects (Huthmacher et al., 2010). These, and other studies, have resulted in a considerable array of approaches to inhibit RNR, which we briefly describe next.

Methods of RNR inhibition

RNR may be targeted at the translational or protein levels. RNR inhibitors are loosely categorized as those that prohibit the formation of an active holoenzyme or those that inhibit the

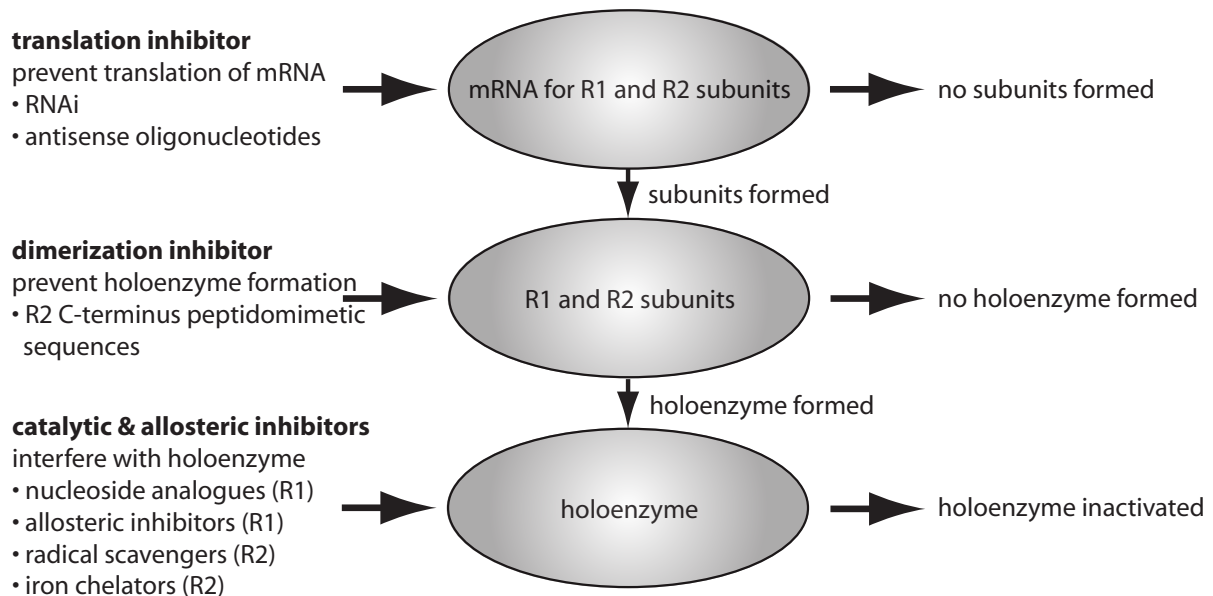


Figure 2. Means of RNR inhibition. A flowchart showing the progression from mRNA to the formation of the holoenzyme (ovals) and how translation, dimerization and catalytic and allosteric inhibitors act along this process.

function of an already fully-formed holoenzyme (Cerqueira et al., 2007). At the translation level, synthesis of the enzyme subunits is blocked, while at the protein level, inhibitors may be employed to prevent the formation of the holoenzyme or inhibitors may be used to inactivate either the R1 or R2 subunit, or both (Cerqueira et al., 2005) (Figure 2). The use of ribozymes, single strand antisense oligonucleotides, and small interfering RNA (siRNA) can all be defined as anti-mRNA strategies, or subunit synthesis inhibitors (see (Aboul-Fadl, 2005) for a review of the implementation, optimization, and practical application of these methodologies), while dimerization, catalytic, and allosteric inhibitors focus on the inhibition of formed proteins.

Subunit synthesis inhibitors

Ribozymes

Ribozymes are catalytic RNA molecules with distinct three-dimensional configurations, which principally exhibit trans-cleavage properties. Ribozymes can be specifically designed to cleave a targeted RNA sequence, thereby inactivating gene transcripts (Haseloff and Gerlach, 1988; Norris et al., 2000; Citti and Rainaldi, 2005). Ribozymes and their applications have been extensively reviewed (Puerta-Fernández et al., 2003; Nayak and Kohli, 2005; Khan, 2006; Tedeschi et al., 2009). Ribozymes offer a productive avenue for gene therapy and have been designed for use against inborn metabolic disorders, viral infections, and cancer (Lewin and Hauswirth, 2001).

Both *in vitro* and *in vivo* studies demonstrated promising use of ribozymes to target the *survivin* gene, which when expressed, leads to cell proliferation, typical in most human carcinomas (Choi et al., 2003). Ribozymes designed to target mouse telomerase RNA were successfully administered and systemically expressed *in vivo*, and served to reduce the metastatic progression of B16_F10 murine melanoma metastases (Nosrati et al., 2004). *In vitro* targeting of RhoC by ribozymes showed reduction of invasiveness in human breast cancer cells and thus demonstrated the utility of ribozymes in gene therapy (Lane et al., 2010). Similarly, ribozymes designed for targeting specific sites for cleavage in human telomerase RNA were demonstrated to be effective in arresting cell growth and induction of spontaneous cell apoptosis in colon cancer cells (Lu et al., 2011).

In the context of apicomplexan control, ribozymes were successfully used to reduce malarial viability up to 55% when targeting *P. falciparum*-specific inserts in the carbamoyl-phosphate II synthetase gene (Flores et al., 1997). Accordingly, C-terminus insertions present in the R1 subunit enzyme of *Plasmodium* and *Theileria* offer sites uniquely different from those of their hosts, which may be specifically targeted by ribozymes (Ingram and Kinnaird, 1999).

RNA interference

RNA interference (RNAi) utilizes segments of double-stranded RNA to interfere with gene expression and it usually relies on the enzyme Dicer and the RNA-induced silencing complex (Scherr et al., 2004). Theoretically, the chemotherapeutic applicability of RNAi, and that of variants on the RNAi theme, is extensive; however, caution is advised as there are safety and specificity concerns (Grimm and Kay, 2007). There have been recent advances in the reduction of non-target effects and improved specificity in

the silencing of target genes with chemically synthesized small interfering RNA (siRNA) (Lee and Sinko, 2006; Vaish et al., 2010), which typically utilizes much shorter lengths of double stranded RNA (20 to 25 bp). siRNA has proven useful in the suppression of p53R2 expression, leading to the inhibition of tumor growth and an increase in sensitivity to anticancer drugs (Yanamoto et al., 2005).

Antisense RNA has been documented in *Plasmodium* (Militello et al., 2005) and there are numerous examples where RNAi has reportedly been successfully used to silence genes in *Plasmodium* (Kumar et al., 2002; Malhotra et al., 2002; McRobert and McConkey, 2002; Mohammed et al., 2003; Dasaradhi et al., 2005; Gissot et al., 2005; Crooke et al., 2006; Sunil et al., 2008; Tuteja et al., 2008; Sriwilaijaroen et al., 2009). However, in stark contrast to these findings, are those where support for RNAi in *Plasmodium* is lacking. In fact, using both an experimental and bioinformatics approach, Baum et al. (Baum et al., 2009) suggested that *Plasmodium* lacks RNAi functionality and the conserved enzymes necessary for RNAi activity such as Dicer and Argonaute-like proteins, or their analogs. It has further been suggested that documented RNAi activity in *Plasmodium* may be the result of general toxicity to the introduced RNA, or an alternative (non-RNAi) antisense mechanism, and not the result of specific gene targeting by RNAi (Ullu et al., 2004). Additional authors have also questioned RNAi activity or the presence of a classical RNAi pathway in Apicomplexa, particularly in *Plasmodium* (Aravind et al., 2003; Blackman, 2003; Cerutti and Casas-Mollano, 2006; Xue et al., 2008). Further calling into question the utility of RNAi is the fact that these studies show down-regulation, but not the elimination, of gene function, and the degree to which protein expression is suppressed depends of a variety of factors (Brown and Catteruccia, 2006).

RNA antisense oligonucleotide inhibitors

Antisense oligonucleotides (AOs) are short (10 to 30 nucleotides), single strands of RNA or DNA that serve to inhibit gene expression. The sequence of an AO is complementary to a chosen target mRNA sequence, to which it will bind via canonical Watson-Crick base pairing. A variety of modifications to antisense oligonucleotides may be employed to prevent degradation, increase affinity and potency, and to reduce non-target toxicity (Chan et al., 2006; Sahu et al., 2007; Li et al., 2010). Further improvements come in terms of selective delivery systems for oligonucleotides (Ming et al., 2010). AOs may knockdown a target mRNA molecule by means of three distinct processes: (1) steric inhibition, where protein translation is inhibited once AOs are bound to the target mRNA, (2) the non-specific endonuclease, ribonuclease H (RNase H), may be activated and catalyze the cleavage of a DNA/mRNA duplex; alternatively, (3) pre-mRNA targeting, which includes inhibition of splicing, inhibition of the 5' cap formation, or de-stabilization of the pre-mRNA, would inhibit mRNA maturation (Ho et al., 1996; Baker and Monia, 1999; Achenbach et al., 2003; Sahu et al., 2007).

Genes encoding both the large R1 RNR subunit and small R2 subunit have been targeted with antisense inhibition. Inhibition of expression of the *Herpes simplex* virus was achieved using AOs to target the R1 translation initiation site (Aurelian and Smith, 2000). The R2-directed AO, GTI-2040, has shown promising selectivity and specificity in its

antitumor activity against a variety of human cancers (Lee et al., 2003; Desai et al., 2005). AOs were designed to target both the R1 and R2 subunits in oropharyngeal KB cancer cells; however, only the targeted inhibition of R2 expression reduced enzyme activity and inhibited cell growth (Chen et al., 2000).

AOs have also proven to be effective against a variety of gene products in *Plasmodium* (Rapaport et al., 1992; Barker et al., 1998; Gardiner et al., 2000; Patankar et al., 2001; Kyes et al., 2002; Noonpakdee et al., 2003; Gunasekera et al., 2004). RNR activity was inhibited by targeting the region surrounding the translation initiation codon with AO phosphorothioates for the *P. falciparum* R2_e1 subunit, (Chakrabarti et al., 1993).

Protein inhibitors

Dimerization (polymerization) inhibitors

Dimerization inhibitors bind to one or more partners in a protein-protein interaction and hence prevent formation of the holoenzyme. In the case of RNR, they are small peptidomimetic sequences that mimic the R2 subunit C-terminal residues. As such, they competitively bind to the hydrophobic cleft in the R1 subunit, to the exclusion of R2, and prevent formation of the holoenzyme (Paradis et al., 1988; Gaudreau et al., 1990; Yang et al., 1990; Cosentino et al., 1991). It is the differences between host and parasite R2 C-terminal sequences that have so far lent themselves to specific targeting of a parasite's RNR.

RNR enzyme activity was inhibited in the *Herpes simplex* virus with the introduction of a peptide that corresponded to the C-terminus amino acid residues of the viral R2 subunit (Gaudreau et al., 1992; Liuzzi et al., 1994). In addition to the *Herpes simplex* virus, RNR dimerization inhibitors have been extensively studied in *E. coli*, hamster, mouse, yeast, and human cells (Cohen et al., 1986; Dutia et al., 1986; Climent et al., 1991; Cosentino et al., 1991; Fisher et al., 1993; Davis et al., 1994).

Likewise, in the case of apicomplexans, it is the difference in the C-terminal sequences of the R2 subunits between parasites and their hosts that may be best exploited by chemotherapeutic means (Chakrabarti et al., 1993; Fisher et al., 1993; Cerqueira et al., 2005). Peptidomimetic inhibitors based on the C-terminus of the small subunit have been proposed as a means of disrupting the formation of the RNR heterodimer complex in *P. falciparum* (Bracchi-Ricard et al., 2005). In fact, targeting malarial RNR activity of *P. falciparum*-infected erythrocytes was accomplished by use of synthetic peptidomimetic peptides, which prevented binding of the R1 and R2 subunits (Rubin et al., 1993).

Catalytic and allosteric inhibitors

Catalytic protein inhibitors target the active RNR holoenzyme and may act on either the R1 or R2 subunits, or both. Catalytic inhibitors may function by a variety of means, be it: (1) the destruction of the R2 subunit radical by radical scavengers or iron chelators, (2) inactivation of the R1 subunit active site, or (3) via substrate/nucleoside analogs, thus primarily acting on the R1 subunit. Allosteric inhibitors, which are also nucleoside analogs, target the R1 effector binding sites.

Radical scavengers such as hydroxyurea, irreversibly destroy the tyrosol radical of the R2 protein (Krakoff et al., 1968; Lepoivre et al., 1991; Szekeres et al., 1997; Fontecave

et al., 1998; Guittet et al., 1999). Hydroxyurea was shown to stop DNA synthesis in *P. falciparum* (Inselburg and Banyal, 1984). Improved control of malaria utilizing a combination therapy of erythropoietin and iron sulfate in conjunction with hydroxyurea has been hypothesized (Saei and Ahmadian, 2009). In contrast to the scavenging of radicals, iron chelators, which may destroy or prevent the formation of the radical (Nyholm et al., 1993; Richardson, 2002; Hodges et al., 2004; Whitnall et al., 2006), do not necessarily cause permanent inhibition. Iron chelators target cellular iron, leading to iron deprivation, which has been suggested to result in RNR inhibition (Pradines et al., 1996). Iron chelators have been demonstrated to be effective against the *P. falciparum* trophozoite and ring stages, which, unlike host cells, demonstrated a limited to irreversible loss of capacity for recovery after the chelator was removed (Lytton et al., 1994).

Substrate analogs are also referred to as suicide inhibitors and were recently reviewed (Perez et al., 2010). They are recognized as "normal" ribonucleotide substrates; however, their interaction with the holoenzyme's active site leads to inactivation of the enzyme. A case in point is inactivation of the R1 active site by use of nucleoside-diphosphate analogs, which bind and result in alkylation of the protein (Pereira et al., 2004; Pereira et al., 2006). Note that some substrate analogs such as gemcitabine and tezacitabine have additional inhibitory effects on the R2 subunit (Salowe et al., 1993; Shao et al., 2006).

As noted earlier, nucleoside-triphosphates bind to the allosteric effector sites and serve to either activate or inhibit the reduction of nucleoside diphosphates (Thelander and Reichard, 1979). Allosteric effector analogs, which are typically nucleoside-triphosphate analogs, thus interact with the two R1 allosteric effector-binding sites, i.e. the A- and S-sites. dATP normally acts as an inhibitor; however, some deoxyadenosine analogs have an even more powerful affect due to their higher affinity for the R1 effector site (Cory and Mansell, 1975; Harrington and Spector, 1991; Jeha et al., 2004; Shao et al., 2006). Interference of the allosteric binding sites will have an influence on the activity and substrate binding affinity of the R1 subunit. While the structure of the allosteric sites in the R1 subunit may be similar between the RNR of an eukaryotic parasite and that of its host, there are usually unique substitutions between the two, which in turn may lead to differences in allosteric regulation. That is the case of the RNR of humans and of *Trypanosoma brucei*, the causative agent of sleeping sickness (Hofer et al., 1997). Chakrabarti et al. (Chakrabarti et al., 1993) suggested that differences in the N-terminus sequence of the R1 subunit of *P. falciparum* might indicate that it too utilizes a different allosteric regulation mechanism relative to humans. The authors suggested that conservation across other protozoans, in terms of the residues whose function it is to bind dTTP, indicates that they too may employ an allosteric regulation mechanism that differs from mammalian hosts. Such a difference has the potential to be exploited via suicide substrate inhibitors or nucleoside analogues (Ingram and Kinnaird, 1999).

Challenges to using RNR to control apicomplexan parasites

First and foremost, the function of the apicomplexan-specific R2_e2 subunit remains unknown and, in particular, whether

this subunit is essential to the formation of a functional RNR holoenzyme has yet to be determined. Munro et al. (submitted) have identified considerable variability among apicomplexans in the amino acid residue that purportedly harbors the free radical in the R2_e2 subunit, as well as in additional residues of functional importance. For example, the lack of conservation of one of the two phenylalanine residues in the C-terminus (Figure 3A) raises the possibility of a difference in the interaction between the R2_e2 and R1 subunits relative to that observed with R2_e1. Following the residue notation of Fisher et al. (Fisher et al., 1993), the C-terminal residues F¹ and F⁷ appear to be particularly influential in dictating the interaction/binding-specificity to subsites of the R1 subunit (Pellegrini et al., 2000; Pender et al., 2001). While the R2_e2 C-terminal residue equivalent to F¹ is maintained as phenylalanine and conserved across R2_e2, the residue equivalent to F⁷ is instead substituted for isoleucine in all sequences sampled, save the cryptosporidians. This may, however, not be a concern; it has been established that F⁷ need not be stringently conserved because the R1 subsite interacting with this R2 subunit position can accommodate a variety of hydrophobic residues (Pender et al., 2001; Gao et al., 2002; Cooperman, 2003). Also, Tyr370 in mouse R2 was determined to be essential in the radical transport pathway (Rova et al., 1999) and yet an equivalent to this residue is lacking in the apicomplexan-specific R2_e2 subunit.

There are further challenges to RNR chemotherapy. Two widely used anti-cancer RNR inhibitors, hydroxyurea and gemcitabine, are toxic to humans (Banach and Williams, 2000; Santini et al., 2000). Additionally, resistance to RNR inhibition by some radical scavengers and iron chelators has been observed (Nocentini et al., 1990; Sneed and Loeb, 2004; Fu and Xiao, 2006). Further concerns with regard to the use of ribozymes include method of delivery, ribozyme stability, the secondary and tertiary structure of the target mRNA, and how these factors relate to accessibility of the region being targeted for cleavage (Turner, 2000).

Bolstering support for use of RNR inhibition to control apicomplexan parasites

Since its discovery in 1961 (Reichard et al., 1961), ribonucleotide reductase has been featured in almost 5,500 publications in a variety of fields, such as biochemistry, molecular biology, oncology, cell biology, and chemistry. As such, there is a wealth of information regarding this protein. The literature is replete with studies of RNR inhibitor use in the control of cancer and of human pathogens. As detailed earlier, RNR has also received attention for its potential use as a target to control the apicomplexan parasite *P. falciparum*. RNR inhibitors have already been shown to have some antimalarial activity. Examples include RNA antisense oligonucleotide inhibitors (Chakrabarti et al., 1993) and radical scavengers such as the substituted/modified benzohydroxamic acids, specifically the vicinal dihydroxybenzohydroxamates (Holland et al., 1998).

Knowledge of protein structure and localization can greatly aid in the design of chemotherapeutic drugs. For example, it is essential to determine if the region being targeted is exposed on the protein's surface, whether it is functional, and whether the residues surrounding the target region in its native conformation are similarly conserved (Durand et al., 2008). While the structure of the

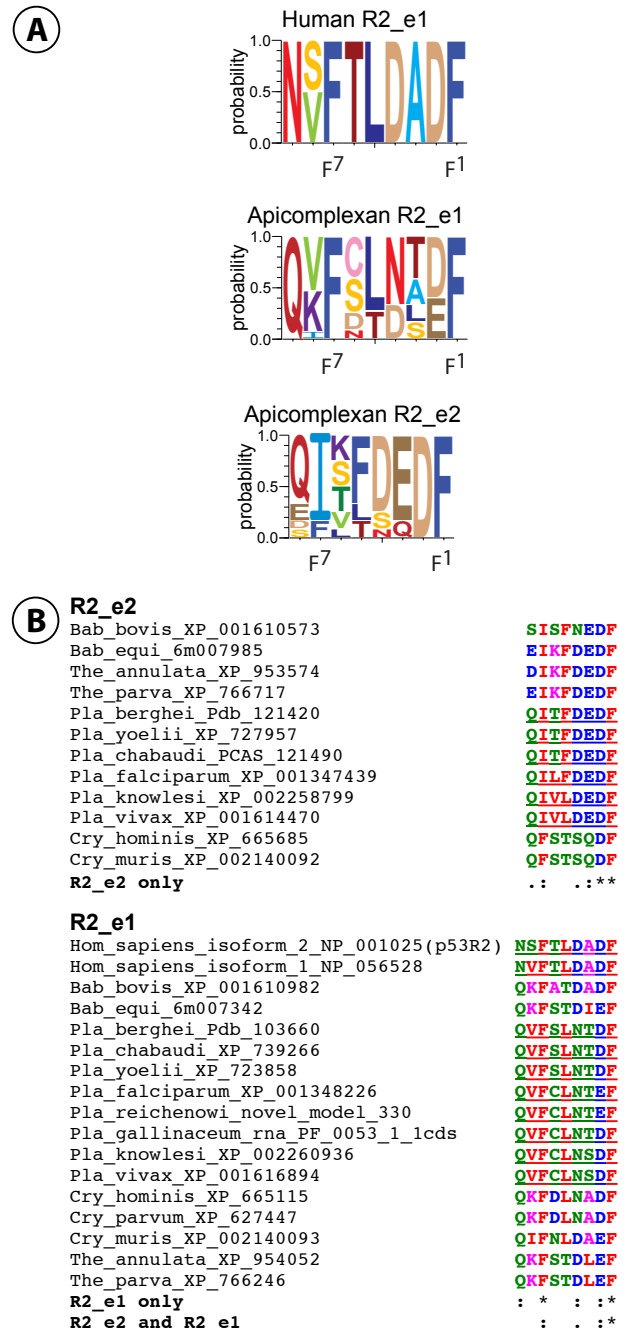


Figure 3. RNR subunit R2 C-terminus. A) Sequence logo representation of human and apicomplexan R2_e1 and apicomplexan R2_e2 terminal residues. F¹ and F⁷ are crucial phenylalanine residues that interact with the R1 subunit. The numbering of these residues follows (Fisher et al., 1993), while subsequent authors appear to have reversed the order (Pellegrini et al., 2000; Pender et al., 2001; Gao et al., 2002; Cooperman, 2003). Created with WebLogo 3 (Schneider and Stephens, 1990; Crooks et al., 2004). B) Terminal residues for human and apicomplexan R2_e1 and apicomplexan R2_e2 from which the logos were derived. Data derived from Munro et al. (submitted). *Plasmodium* and human sequences are underlined for comparative purposes (see text). Amino acid substitutions: . = semiconserved, : = conserved, * = identical.

apicomplexan-specific R2 subunit is not known, structure of the orthodox R2_e1 subunit from *Plasmodium vivax* (2O1Z) and *P. yoelii* (2P11) are deposited on the RCSB Protein Data Bank (www.pdb.org; Berman et al., 2000).

Advances have been made in the production of apicomplexan recombinant proteins, a process that has been historically hampered by the A+T-biased nature of the plasmodial genome and uncommon codon usage (Weber, 1987; Anonymous, 2006; Brombacher 2006). However, see Vedadi et al. (Vedadi et al., 2007) who found *E. coli* to effectively produce apicomplexan proteins on a large-scale basis. Codon optimization (Hedfalk et al., 2008) and codon harmonization (Hillier et al., 2005; Angov et al., 2008; Chowdhury et al., 2009) have been used to improve expression of *Plasmodium* proteins in *E. coli*. Further advances have been made in the area of the use of a phylogenetically similar, or “pseudoparasite”, expression system (Fernández-Robledo and Vasta, 2010). Furthermore, *in vitro* protocols for *P. falciparum* are established, although *in vivo* animal models are based on the murine *Plasmodium* species *P. berghei*, *P. chabaudi*, *P. vinkei*, and *P. yoelii*, and not those that parasitize humans (Fidock et al., 2004).

To a large degree, the utility of RNR as an antimalarial chemotherapeutic target is dependent upon the timing of the protein's expression. In mammals and yeast, the large subunit R1 has a half-life of 24 hours and is maintained at a constant level throughout a cell's life cycle, while the small subunit R2 has a half-life of around 3 hours and its expression is restricted from the S-phase through to late mitosis, at which time it is rapidly degraded (Eriksson et al., 1984; Engström et al., 1985; Björklund et al., 1990; Elledge et al., 1992). The non-canonical human p53R2 protein is expressed during periods of DNA repair (Håkansson et al., 2006). In mammalian cells, it has been demonstrated that it is the presence or absence of the R2 protein that regulates RNR activity (Chabes and Thelander, 2000). In contrast to this, in *S. cerevisiae* it is the R1 subunit whose transcription is increased during S-phase, thus controlling RNR activity (Ortigosa et al., 2006). The utility of RNR inhibition in the control of certain cancers has relied in part on the fact that RNR is most needed when cells are rapidly proliferating, rendering cancerous cells particularly vulnerable to RNR inhibition (Smith and Karp, 2003). In *P. falciparum*, ribonucleotide biosynthesis begins as early as the ring and early trophozoite stage; however, deoxyribonucleotide metabolism occurs later, with both R1 and R2_e1 subunit transcripts detected in the red blood cells (RBCs) at 10 hours post RBC infection and peaking 31 to 33 hours post-infection, which coincides with the late trophozoite/early schizont stage (Chakrabarti et al., 1993; Bozdech et al., 2003; Bozdech and Ginsburg, 2004). Comprehensive expression studies in *P. falciparum* show that the expression profile of R2_e2, albeit less intense, matches that of the other two subunits (Bozdech et al., 2003; Llinás et al., 2006). The timing of RNR expression is not surprising, since it is during the intraerythrocytic stages that the malarial parasite undergoes logarithmic growth and requires RNR for DNA synthesis (Yeh and Altman, 2006). Additionally, expression of PfR4 (R2_e2) has been detected in the sporozoite and gametocyte stages of the parasite life cycle (Bracchi-Ricard et al., 2005), and in the case of *P. yoelii*, both R1 and R2_e1 transcripts were detected in the liver stage of infection (Nivez et al., 2000). These expression profiles are well-

suited for chemotherapeutic intervention, since it is the intraerythrocytic stages of *Plasmodium* that cause clinical symptoms (Yeh and Altman, 2006). Additionally, human RBCs are not nucleated, thus precluding an alternative means for the parasite to exploit host RNR (Rubin et al., 1993).

The utility of RNR as an antimalarial/antipathogen target depends upon the ability to specifically target the pathogen's RNR and not that of the host, as RNR is essential to both species. R1 and R2 subunits are highly conserved between prokaryotes and eukaryotes in the regions containing the functionally important residues (Chakrabarti et al., 1993; Roshick et al., 2000; Voegtli et al., 2001; Högbom et al., 2004; Högbom, 2010); however, they differ in the N- and C-terminal sequences (Ingram and Kinnaird, 1999). Novel RNRs are additional potential targets for new drugs, especially if they provide distinct differences between host and parasite sequences. The necessity for target specificity to avoid side, or non-target, effects in humans cannot be overstated. The use of antisense oligonucleotides in the control of some cancers has shown that the drugs in question have favorable toxicity profiles, in part because of their ability to specifically target segments of RNA (Davies et al., 2003). The unorthodox R2_e2 apicomplexan subunit provides a distinct and additional opportunity for specific drug targeting. One example is the C-terminus of the R2 subunit, which differs considerably between the human R2_e1 subunits and both the R2_e1 and R2_e2 apicomplexan subunits (Figures 3A and 3B). This difference in the C-terminal sequences of the R2 subunits between apicomplexans and their hosts can be ideally targeted by chemotherapeutic means (Rubin et al., 1993; Fisher et al., 1995; Ingram and Kinnaird, 1999).

Finally, it is worth noting that *Plasmodium*-infected erythrocytes demonstrate an increase in cell membrane permeability (Baumeister et al., 2011). *In vitro* uptake of small pieces of RNA becomes less of an issue as RBCs that are infected with malaria exhibit an enhanced and selective uptake of such molecules in comparison to non-infected RBCs (Rapaport et al., 1992), a difference attributed to the presence of a parasitophorous ducts in infected RBCs (Pouvelle et al., 1991).

Summary

Extensive research has already established that RNR has potential as an antimalarial drug. What is particularly appealing about RNR inhibition as a means of controlling Apicomplexa is the potential control of not one, but two, copies of the R2 subunit, both of which are distinct from that of the host. Additionally, in the case of the malarial parasite *Plasmodium*, RNR expression occurs from the sporozoite through the gametocyte life cycle stage, offering multiple opportunities for chemotherapeutic targeting. This may well be the case for other Apicomplexa parasites that undergo rapid clonal expansion in the host.

Of the eight established methods of RNR inhibition discussed, RNAi seems the least promising in terms of controlling apicomplexan parasites as the necessary enzymes appear to be lacking. Ribozyme approaches have been successfully implemented in *Plasmodium*; however their use against RNR has not yet been demonstrated. In contrast, substrate analogs and allosteric effector analogs have been effectively used to inhibit RNR, but their use

in *Plasmodium* has yet to be demonstrated. Antisense oligonucleotide inhibitors, dimerization inhibitors, radical scavengers, and iron chelators have all been successfully used to target RNR in *Plasmodium*. Ribozymes, antisense oligonucleotide inhibitors, and dimerization inhibitors show the most promise in terms of future anti-apicomplexan drug development. On the other hand, resistance to some radical scavengers and iron chelators has been established, they can not be used to selectively target a peptide, and the fact that the effects of iron chelators are generally reversible, makes them less appealing prospects.

The difference in sequence similarity between the parasite and human R2_e1 subunits is considerable, and the difference is even more accentuated when the parasite's R2_e2 subunit is considered (Munro et al., submitted). Assuming that this protein is essential for RNR function, ribozymes, antisense oligonucleotide inhibitors, and dimerization inhibitors can all be optimized for target specificity and thus used to take advantage of the unique R2_e2 protein. The fact that the R2_e2 gene is present in several apicomplexan genera, each of which contains species of significant health and socio-economic impact, holds promise that the results of any research would be translatable to several very important diseases.

References

- Aboul-Fadl, T. (2005). Antisense oligonucleotides: The state of the art. *Curr Med Chem* 12, 2193-2214.
- Achenbach, T.V., Brunner, B., and Heermeier, K. (2003). Oligonucleotide-based knockdown technologies: Antisense versus RNA interference. *ChemBioChem* 4, 928-935.
- Adl, S.M., Simpson, A.G.B., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., et al. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* 52, 399-451.
- Akiyoshi, D.E., Balakrishnan, R., Huettinger, C., Widmer, G., and Tzipori, S. (2002). Molecular characterization of ribonucleotide reductase from *Cryptosporidium parvum*. *DNA Sequence* 13, 167-172.
- Alonso, P.L., Djimde, A., Kremsner, P., Magill, A., Milman, J., Nájera, J., Plowe, C.V., Rabinovich, R., Wells, T., and Yeung, S. (2011). A research agenda for malaria eradication: Drugs. *PLoS Medicine* 8, e1000402.
- Anderson, T. (2009). Mapping the spread of malaria drug resistance. *PLoS Medicine* 6, e1000054.
- Andersson, C.S., and Högbom, M. (2009). A *Mycobacterium tuberculosis* ligand-binding Mn/Fe protein reveals a new cofactor in a remodeled R2-protein scaffold. *Proc Natl Acad Sci Unit States Am* 106, 5633-5638.
- Angov, E., Hillier, C.J., Kincaid, R.L., and Lyon, J.A. (2008). Heterologous protein expression is enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host. *PLoS One* 3, e2189.
- Anonymous (2006). Crystal ball. *Parasite Immunol* 28, 235-269.
- Aravind, L., Iyer, L.M., Wellems, T.E., and Miller, L.H. (2003). *Plasmodium* biology: Genomic gleanings. *Cell* 115, 771-785.
- Aurelian, L., and Smith, C.C. (2000). Herpes simplex virus type 2 growth and latency reactivation by cocultivation are inhibited with antisense oligonucleotides complementary to the translation initiation site of the large subunit of ribonucleotide reductase (RR1). *Antisense Nucleic Acid Drug Dev* 10, 77-85.
- Baker, B.F., and Monia, B.P. (1999). Novel mechanisms for antisense-mediated regulation of gene expression. *Biochim Biophys Acta* 1489, 3-18.
- Banach, M.J., and Williams, G.A. (2000). Purtscher retinopathy and necrotizing vasculitis with gemcitabine therapy. *Arch Ophthalmol* 118, 726-727.
- Barker, R.H., Metelev, V., Coakley, A., and Zamecnik, P. (1998). *Plasmodium falciparum*: Effect of chemical structure on efficacy and specificity of antisense oligonucleotides against malaria *in vitro*. *Exp Parasitol* 88, 51-59.
- Barker, R.H., Metelev, V., Rapaport, E., and Zamecnik, P. (1996). Inhibition of *Plasmodium falciparum* malaria using antisense oligodeoxynucleotides. *Proc Natl Acad Sci Unit States Am* 93, 514-518.
- Baum, J., Papenfuss, A.T., Mair, G.R., Janse, C.J., Vlachou, D., Waters, A.P., Cowman, A.F., Crabb, B.S., and de Koning-Ward, T.F. (2009). Molecular genetics and comparative genomics reveal RNAi is not functional in malaria parasites. *Nucleic Acids Res* 37, 3788-3798.
- Baumeister, S., Wiesner, J., Reichenberg, A., Hintz, M., Bietz, S., Harb, O.S., Roos, D.S., Kordes, M., Friesen, J., Matuschewski, K., et al. (2011). Fosmidomycin uptake into *Plasmodium* and *Babesia*-infected erythrocytes is facilitated by parasite-induced new permeability pathways. *PLoS One* 6, e19334.
- Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., and Bourne, P.E. (2000). The Protein Data Bank. *Nucleic Acids Res* 28, 235-242.
- Bianchi, V., Borella, S., Calderazzo, F., Ferraro, P., Chieco Bianchi, L., and Reichard, P. (1994). Inhibition of ribonucleotide reductase by 2'-substituted deoxycytidine analogs: Possible application in AIDS treatment. *Proc Natl Acad Sci Unit States Am* 91, 8403-8407.
- Björklund, S., Skog, S., Tribukait, B., and Thelander, L. (1990). S-phase-specific expression of mammalian ribonucleotide reductase R1 and R2 subunit mRNAs. *Biochemistry* 29, 5452-5458.
- Blackman, M.J. (2003). RNAi in protozoan parasites: What hope for the Apicomplexa? *Protist* 154, 177-180.
- Blanchard, J.L., and Hicks, J.S. (1999). The non-photosynthetic plastid in malarial parasites and other apicomplexans is derived from outside the green plastid lineage. *J Eukaryot Microbiol* 46, 367-375.
- Bledsoe, G.H. (2005). Malaria primer for clinicians in the United States. *South Med J* 98, 1197-1204; quiz 1205, 1230.
- Booden, T., and Hull, R.W. (1973). Nucleic acid precursor synthesis by *Plasmodium lophurae* parasitizing chicken erythrocytes. *Exp Parasitol* 34, 220-228.
- Bourdon, A., Minai, L., Serre, V., Jais, J.-P., Sarzi, E., Aubert, S., Chrétien, D., De Lonlay, P., Paquis-Flucklinger, V., Arakawa, H., et al. (2007). Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. *Nat Genet* 39, 776-780.

- Bozdech, Z., and Ginsburg, H. (2004). Antioxidant defense in *Plasmodium falciparum*—data mining of the transcriptome. *Malaria Journal* 3, 23.
- Bozdech, Z., Llinás, M., Pulliam, B.L., Wong, E.D., Zhu, J., and DeRisi, J.L. (2003). The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. *PLoS Biol* 1, E5.
- Bracchi-Ricard, V., Moe, D., and Chakrabarti, D. (2005). Two *Plasmodium falciparum* ribonucleotide reductase small subunits, Pfr2 and Pfr4, interact with each other and are components of the *in vivo* enzyme complex. *J Mol Biol* 347, 749-758.
- Brombacher, F. (2006). Crystal ball. *Parasite Immunol* 28, 235-269.
- Brown, A.E., and Catteruccia, F. (2006). Toward silencing the burden of malaria: Progress and prospects for RNAi-based approaches. *BioTechniques Suppl*, 38-44.
- Brown, N.C., and Reichard, P. (1969). Role of effector binding in allosteric control of ribonucleoside diphosphate reductase. *J Mol Biol* 46, 39-55.
- Bustamante, C., Batista, C.N., and Zalis, M. (2009). Molecular and biological aspects of antimalarial resistance in *Plasmodium falciparum* and *Plasmodium vivax*. *Curr Drug Targets* 10, 279-290.
- Cao, M.-Y., Lee, Y., Feng, N.-P., Xiong, K., Jin, H., Wang, M., Vassilakos, A., Viau, S., Wright, J.A., and Young, A.H. (2003). Adenovirus-mediated ribonucleotide reductase R1 gene therapy of human colon adenocarcinoma. *Clin Cancer Res* 9, 4553-4561.
- Carvalho, T.G., and Ménard, R. (2005). Manipulating the *Plasmodium* genome. *Curr Issues Mol Biol* 7, 39-55.
- Cassera, M.B., Hazleton, K.Z., Riegelhaupt, P.M., Merino, E.F., Luo, M., Akabas, M.H., and Schramm, V.L. (2008). Erythrocytic adenosine monophosphate as an alternative purine source in *Plasmodium falciparum*. *J Biol Chem* 283, 32889-32899.
- Cerqueira, N.M.F.S.A., Fernandes, P.A., and Ramos, M.J. (2007). Ribonucleotide reductase: A critical enzyme for cancer chemotherapy and antiviral agents. *Recent Pat Anti-Canc* 2, 11-29.
- Cerqueira, N.M.F.S.A., Pereira, S., Fernandes, P.A., and Ramos, M.J. (2005). Overview of ribonucleotide reductase inhibitors: An appealing target in anti-tumour therapy. *Curr Med Chem* 12, 1283-1294.
- Cerutti, H., and Casas-Mollano, J.A. (2006). On the origin and functions of RNA-mediated silencing: From protists to man. *Curr Genet* 50, 81-99.
- Chabes, A., Domkin, V., Larsson, G., Liu, A., Graslund, A., Wijmenga, S., and Thelander, L. (2000). Yeast ribonucleotide reductase has a heterodimeric iron-radical-containing subunit. *Proc Natl Acad Sci Unit States Am* 97, 2474-2479.
- Chabes, A., Georgieva, B.P., Domkin, V., Zhao, X., Rothstein, R., and Thelander, L. (2003). Survival of DNA damage in yeast directly depends on increased dNTP levels allowed by relaxed feedback inhibition of ribonucleotide reductase. *Cell* 112, 391-401.
- Chabes, A., and Thelander, L. (2000). Controlled protein degradation regulates ribonucleotide reductase activity in proliferating mammalian cells during the normal cell cycle and in response to DNA damage and replication blocks. *J Biol Chem* 275, 17747-17753.
- Chakrabarti, D., Schuster, S.M., and Chakrabarti, R. (1993). Cloning and characterization of subunit genes of ribonucleotide reductase, a cell-cycle-regulated enzyme, from *Plasmodium falciparum*. *Proc Natl Acad Sci Unit States Am* 90, 12020-12024.
- Chan, J.H.P., Lim, S., and Wong, W.S.F. (2006). Antisense oligonucleotides: From design to therapeutic application. *Clin Exp Pharmacol Physiol* 33, 533-540.
- Chaudhary, K., Darling, J.A., Fohl, L.M., Sullivan, W.J., Donald, R.G.K., Pfefferkorn, E.R., Ullman, B., and Roos, D.S. (2004). Purine salvage pathways in the apicomplexan parasite *Toxoplasma gondii*. *J Biol Chem* 279, 31221-31227.
- Chen, S., Zhou, B., He, F., and Yen, Y. (2000). Inhibition of human cancer cell growth by inducible expression of human ribonucleotide reductase antisense cDNA. *Antisense Nucleic Acid Drug Dev* 10, 111-116.
- Choi, K.S., Lee, T.H., and Jung, M.H. (2003). Ribozyme-mediated cleavage of the human survivin mRNA and inhibition of antiapoptotic function of survivin in MCF-7 cells. *Cancer Gene Ther* 10, 87-95.
- Chowdhury, D.R., Angov, E., Kariuki, T., and Kumar, N. (2009). A potent malaria transmission blocking vaccine based on codon harmonized full length Pfs48/45 expressed in *Escherichia coli*. *PLoS One* 4, e6352.
- Chrubasik, C., and Jacobson, R.L. (2010). The development of artemisinin resistance in malaria: Reasons and solutions. *Phytother Res* 24, 1104-1106.
- Citti, L., and Rainaldi, G. (2005). Synthetic hammerhead ribozymes as therapeutic tools to control disease genes. *Curr Gene Ther* 5, 11-24.
- Climent, I., Sjöberg, B.-M., and Huang, C.Y. (1991). Carboxyl-terminal peptides as probes for *Escherichia coli* ribonucleotide reductase subunit interaction: Kinetic analysis of inhibition studies. *Biochemistry* 30, 5164-5171.
- Climent, I., Sjöberg, B.-M., and Huang, C.Y. (1992). Site-directed mutagenesis and deletion of the carboxyl terminus of *Escherichia coli* ribonucleotide reductase protein R2. Effects on catalytic activity and subunit interaction. *Biochemistry* 31, 4801-4807.
- Cohen, E.A., Gaudreau, P., Brazeau, P., and Langelier, Y. (1986). Specific inhibition of herpesvirus ribonucleotide reductase by a nonapeptide derived from the carboxy terminus of subunit 2. *Nature* 321, 441-443.
- Coombs, G.H. (1999). Biochemical peculiarities and drug targets in *Cryptosporidium parvum*: Lessons from other coccidian parasites. *Parasitol Today* 15, 333-338.
- Cooperman, B.S. (2003). Oligopeptide inhibition of class I ribonucleotide reductases. *Biopolymers* 71, 117-131.
- Corso, P.S., Kramer, M.H., Blair, K.A., Addiss, D.G., Davis, J.P., and Haddix, A.C. (2003). Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerg Infect Dis* 9, 426-431.
- Cory, J.G. (1988). Ribonucleotide reductase as a chemotherapeutic target. *Adv Enzyme Regul* 27, 437-455.
- Cory, J.G., and Mansell, M.M. (1975). Studies on mammalian ribonucleotide reductase inhibition by pyridoxal phosphate and the dialdehyde derivatives of adenosine, adenosine 5'-monophosphate, and adenosine 5'-triphosphate. *Canc Res* 35, 390-396.

- Cosentino, G., Lavallée, P., Rakhit, S., Plante, R., Gaudette, Y., Lawetz, C., Whitehead, P.W., Ducepe, J.S., Lépine-Frenette, C., and Dansereau, N. (1991). Specific inhibition of ribonucleotide reductases by peptides corresponding to the C-terminal of their second subunit. *Biochem Cell Biol* 69, 79-83.
- Coulson, R.M.R., Hall, N., and Ouzounis, C.A. (2004). Comparative genomics of transcriptional control in the human malaria parasite *Plasmodium falciparum*. *Genome Res* 14, 1548-1554.
- Cox-Singh, J., Davis, T.M.E., Lee, K.-S., Shamsul, S.S.G., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J., and Singh, B. (2008). *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis* 46, 165-171.
- Croft, S.L. (1997). The current status of antiparasite chemotherapy. *Parasitology* 114, S3-S15.
- Crona, M., Furrer, E., Torrents, E., Edgell, D.R., and Sjöberg, B.-M. (2010). Subunit and small-molecule interaction of ribonucleotide reductases via surface plasmon resonance biosensor analyses. *Protein Eng Des Sel* 23, 633-641.
- Crooke, A., Diez, A., Mason, P.J., and Bautista, J.M. (2006). Transient silencing of *Plasmodium falciparum* bifunctional glucose-6-phosphate dehydrogenase-6-phosphogluconolactonase. *FEBS J* 273, 1537-1546.
- Crooks, G.E., Hon, G., Chandonia, J.-M., and Brenner, S.E. (2004). WebLogo: a sequence logo generator. *Genome Research* 14, 1188-1190.
- Dahl, E.L., and Rosenthal, P.J. (2008). Apicoplast translation, transcription and genome replication: Targets for antimalarial antibiotics. *Trends Parasitol* 24, 279-284.
- Dahl, E.L., Shock, J.L., Shenai, B.R., Gut, J., DeRisi, J.L., and Rosenthal, P.J. (2006). Tetracyclines specifically target the apicoplast of the malaria parasite *Plasmodium falciparum*. *Antimicrob Agents Chemother* 50, 3124-3131.
- Dasaradhi, P.V.N., Mohammed, A., Kumar, A., Hossain, M.J., Bhatnagar, R.K., Chauhan, V.S., and Malhotra, P. (2005). A role of falcipain-2, principal cysteine proteases of *Plasmodium falciparum* in merozoite egression. *Biochem Biophys Res Comm* 336, 1062-1068.
- Davies, A.M., Gandara, D.R., Lara, P.N., Mack, P.C., Lau, D.H.M., and Gumerlock, P.H. (2003). Antisense oligonucleotides in the treatment of non-small-cell lung cancer. *Clin Lung Canc* 4 Suppl 2, S68-73.
- Davis, R., Thelander, M., Mann, G.J., Behravan, G., Soucy, F., Beaulieu, P., Lavallée, P., Gräslund, A., and Thelander, L. (1994). Purification, characterization, and localization of subunit interaction area of recombinant mouse ribonucleotide reductase R1 subunit. *J Biol Chem* 269, 23171-23176.
- de Koning, H.P., Bridges, D.J., and Burchmore, R.J.S. (2005). Purine and pyrimidine transport in pathogenic protozoa: From biology to therapy. *FEMS Microbiol Rev* 29, 987-1020.
- Desai, A.A., Schilsky, R.L., Young, A., Janisch, L., Stadler, W.M., Vogelzang, N.J., Cadden, S., Wright, J.A., and Ratain, M.J. (2005). A phase I study of antisense oligonucleotide GTI-2040 given by continuous intravenous infusion in patients with advanced solid tumors. *Ann Oncol* 16, 958-965.
- Dharia, N.V., Bright, A.T., Westenberger, S.J., Barnes, S.W., Batalov, S., Kuhen, K., Borboa, R., Federe, G.C., McClean, C.M., Vinetz, J.M., et al. (2010). Whole-genome sequencing and microarray analysis of *ex vivo Plasmodium vivax* reveal selective pressure on putative drug resistance genes. *Proc Natl Acad Sci Unit States Am* 107, 20045-20050.
- Dondorp, A.M., Yeung, S., White, L., Nguon, C., Day, N.P.J., Socheat, D., and von Seidlein, L. (2010). Artemisinin resistance: Current status and scenarios for containment. *Nat Rev Microbiol* 8, 272-280.
- Doolan, D.L., Aguiar, J.C., Weiss, W.R., Sette, A., Felgner, P.L., Regis, D.P., Quinones-Casas, P., Yates, J.R., Blair, P.L., Richie, T.L., et al. (2003). Utilization of genomic sequence information to develop malaria vaccines. *J Exp Biol* 206, 3789-3802.
- Dormeyer, M., Schöneck, R., Dittmar, G.A., and Krauth-Siegel, R.L. (1997). Cloning, sequencing and expression of ribonucleotide reductase R2 from *Trypanosoma brucei*. *FEBS Lett* 414, 449-453.
- Downie, M.J., Kirk, K., and Mamoun, C.B. (2008). Purine salvage pathways in the intraerythrocytic malaria parasite *Plasmodium falciparum*. *Eukaryot Cell* 7, 1231-1237.
- Durand, P.M., Naidoo, K., and Coetzer, T.L. (2008). Evolutionary patterning: A novel approach to the identification of potential drug target sites in *Plasmodium falciparum*. *PLoS One* 3, e3685.
- Dutia, B.M., Frame, M.C., Subak-Sharpe, J.H., Clark, W.N., and Marsden, H.S. (1986). Specific inhibition of herpesvirus ribonucleotide reductase by synthetic peptides. *Nature* 321, 439-441.
- Ekanem, J. (2001). Ribonucleotide reductase as target for drug discovery against African sleeping sickness. *NISEB Journal* 1, 287-292.
- El Bissati, K., Zufferey, R., Witola, W.H., Carter, N.S., Ullman, B., and Ben Mamoun, C. (2006). The plasma membrane permease PfNT1 is essential for purine salvage in the human malaria parasite *Plasmodium falciparum*. *Proc Natl Acad Sci Unit States Am* 103, 9286-9291.
- Elledge, S.J., and Davis, R.W. (1990). Two genes differentially regulated in the cell cycle and by DNA-damaging agents encode alternative regulatory subunits of ribonucleotide reductase. *Gene Dev* 4, 740-751.
- Elledge, S.J., Zhou, Z., and Allen, J.B. (1992). Ribonucleotide reductase: Regulation, regulation, regulation. *Trends Biochem Sci* 17, 119-123.
- Engström, Y., Eriksson, S., Jildevik, I., Skog, S., Thelander, L., and Tribukait, B. (1985). Cell cycle-dependent expression of mammalian ribonucleotide reductase. Differential regulation of the two subunits. *J Biol Chem* 260, 9114-9116.
- Enserink, M. (2010). Malaria's drug miracle in danger. *Science* 328, 844-846.
- Ericsson, D.J., Nurbo, J., Muthas, D., Hertzberg, K., Lindeberg, G., Karlén, A., and Unge, T. (2010). Identification of small peptides mimicking the R2 C-terminus of *Mycobacterium tuberculosis* ribonucleotide reductase. *J Pept Sci* 16, 159-164.
- Eriksson, S., Gräslund, A., Skog, S., Thelander, L., and Tribukait, B. (1984). Cell cycle-dependent regulation of mammalian ribonucleotide reductase. The S phase-correlated increase in subunit M2 is regulated by *de novo* protein synthesis. *J Biol Chem* 259, 11695-11700.

- Eriksson, S., Thelander, L., and Åkerman, M. (1979). Allosteric regulation of calf thymus ribonucleoside diphosphate reductase. *Biochemistry* *18*, 2948-2952.
- Fairman, J.W., Wijerathna, S.R., Ahmad, M.F., Xu, H., Nakano, R., Jha, S., Prendergast, J., Welin, R.M., Flodin, S., Roos, A., *et al.* (2011). Structural basis for allosteric regulation of human ribonucleotide reductase by nucleotide-induced oligomerization. *Nat Struct Biol* *18*, 316-322.
- Fan, H., Huang, A., Villegas, C., and Wright, J.A. (1997). The R1 component of mammalian ribonucleotide reductase has malignancy-suppressing activity as demonstrated by gene transfer experiments. *Proc Natl Acad Sci Unit States Am* *94*, 13181-13186.
- Fan, H., Villegas, C., Huang, A., and Wright, J.A. (1998). The mammalian ribonucleotide reductase R2 component cooperates with a variety of oncogenes in mechanisms of cellular transformation. *Canc Res* *58*, 1650-1653.
- Fast, N.M., Kissinger, J.C., Roos, D.S., and Keeling, P.J. (2001). Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol Biol Evol* *18*, 418-426.
- Fernández-Robledo, J.A., and Vasta, G.R. (2010). Production of recombinant proteins from protozoan parasites. *Trends Parasitol* *26*, 244-254.
- Fidock, D.A. (2010). Drug discovery: Priming the antimalarial pipeline. *Nature* *465*, 297-298.
- Fidock, D.A., Rosenthal, P.J., Croft, S.L., Brun, R., and Nwaka, S. (2004). Antimalarial drug discovery: Efficacy models for compound screening. *Nat Rev Drug Discov* *3*, 509-520.
- Fisher, A., Laub, P.B., and Cooperman, B.S. (1995). NMR structure of an inhibitory R2 C-terminal peptide bound to mouse ribonucleotide reductase R1 subunit. *Nat Struct Biol* *2*, 951-955.
- Fisher, A., Yang, F.D., Rubin, H., and Cooperman, B.S. (1993). R2 C-terminal peptide inhibition of mammalian and yeast ribonucleotide reductase. *J Med Chem* *36*, 3859-3862.
- Flores, M.V., Atkins, D., Wade, D., O'Sullivan, W.J., and Stewart, T.S. (1997). Inhibition of *Plasmodium falciparum* proliferation *in vitro* by ribozymes. *J Biol Chem* *272*, 16940-16945.
- Fontecave, M. (1998). Ribonucleotide reductases and radical reactions. *Cell Mol Life Sci* *54*, 684-695.
- Fontecave, M., Lepoivre, M., Elleingand, E., Gerez, C., and Guittet, O. (1998). Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett* *421*, 277-279.
- Foth, B.J., Ralph, S.A., Tonkin, C.J., Struck, N.S., Fraunholz, M., Roos, D.S., Cowman, A.F., and McFadden, G.I. (2003). Dissecting apicoplast targeting in the malaria parasite *Plasmodium falciparum*. *Science* *299*, 705-708.
- Fu, Y., and Xiao, W. (2006). Identification and characterization of CRT10 as a novel regulator of *Saccharomyces cerevisiae* ribonucleotide reductase genes. *Nucleic Acids Res* *34*, 1876-1883.
- Gao, Y., Liehr, S., and Cooperman, B.S. (2002). Affinity-driven selection of tripeptide inhibitors of ribonucleotide reductase. *Bioorg Med Chem Lett* *12*, 513-515.
- Gardiner, D.L., Holt, D.C., Thomas, E.A., Kemp, D.J., and Trenholme, K.R. (2000). Inhibition of *Plasmodium falciparum* *clag9* gene function by antisense RNA. *Mol Biochem Parasitol* *110*, 33-41.
- Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., *et al.* (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* *419*, 498-511.
- Gaudreau, P., Brazeau, P., Richer, M., Cormier, J., Langlois, D., and Langelier, Y. (1992). Structure-function studies of peptides inhibiting the ribonucleotide reductase activity of herpes simplex virus type I. *J Med Chem* *35*, 346-350.
- Gaudreau, P., Michaud, J., Cohen, E.A., Langelier, Y., and Brazeau, P. (1987). Structure-activity studies on synthetic peptides inhibiting herpes simplex virus ribonucleotide reductase. *J Biol Chem* *262*, 12413-12416.
- Gaudreau, P., Paradis, H., Langelier, Y., and Brazeau, P. (1990). Synthesis and inhibitory potency of peptides corresponding to the subunit 2 C-terminal region of herpes virus ribonucleotide reductases. *J Med Chem* *33*, 723-730.
- Ge, J., Perlstein, D.L., Nguyen, H.H., Bar, G., Griffin, R.G., and Stubbe, J. (2001). Why multiple small subunits (Y2 and Y4) for yeast ribonucleotide reductase? Toward understanding the role of Y4. *Proc Natl Acad Sci Unit States Am* *98*, 10067-10072.
- Gissot, M., Briquet, S., Refour, P., Boschet, C., and Vaquero, C. (2005). PfMyb1, a *Plasmodium falciparum* transcription factor, is required for intra-erythrocytic growth and controls key genes for cell cycle regulation. *J Mol Biol* *346*, 29-42.
- Gon, S., Camara, J.E., Klungsøyr, H.K., Crooke, E., Skarstad, K., and Beckwith, J. (2006). A novel regulatory mechanism couples deoxyribonucleotide synthesis and DNA replication in *Escherichia coli*. *EMBO J* *25*, 1137-1147.
- Greenwood, B., and Mutabingwa, T. (2002). Malaria in 2002. *Nature* *415*, 670-672.
- Gregson, A., and Plowe, C.V. (2005). Mechanisms of resistance of malaria parasites to antifolates. *Pharmacol Rev* *57*, 117-145.
- Grimm, D., and Kay, M.A. (2007). Therapeutic application of RNAi: Is mRNA targeting finally ready for prime time? *J Clin Invest* *117*, 3633-3641.
- Guittet, O., Håkansson, P., Voevodskaya, N., Fridd, S., Gräslund, A., Arakawa, H., Nakamura, Y., and Thelander, L. (2001). Mammalian p53R2 protein forms an active ribonucleotide reductase *in vitro* with the R1 protein, which is expressed both in resting cells in response to DNA damage and in proliferating cells. *J Biol Chem* *276*, 40647-40651.
- Guittet, O., Roy, B., and Lepoivre, M. (1999). Nitric oxide: A radical molecule in quest of free radicals in proteins. *Cell Mol Life Sci* *55*, 1054-1067.
- Gunasekera, A.M., Patankar, S., Schug, J., Eisen, G., Kissinger, J.C., Roos, D., and Wirth, D.F. (2004). Widespread distribution of antisense transcripts in the *Plasmodium falciparum* genome. *Mol Biochem Parasitol* *136*, 35-42.
- Gwilt, P.R., and Tracewell, W.G. (1998). Pharmacokinetics and pharmacodynamics of hydroxyurea. *Clin Pharmacokinet* *34*, 347-358.
- Håkansson, P., Hofer, A., and Thelander, L. (2006). Regulation of mammalian ribonucleotide reduction and dNTP pools after DNA damage and in resting cells. *J Biol Chem* *281*, 7834-7841.

- Harder, J. (1993). Ribonucleotide reductases and their occurrence in microorganisms: A link to the RNA/DNA transition. *FEMS Microbiol Rev* 12, 273-292.
- Harrington, J.A., and Spector, T. (1991). Human ribonucleotide reductase. Activation and inhibition by analogs of ATP. *Biochem Pharmacol* 42, 759-763.
- Haseloff, J., and Gerlach, W.L. (1988). Simple RNA enzymes with new and highly specific endoribonuclease activities. *Nature* 334, 585-591.
- Hedfalk, K., Pettersson, N., Oberg, F., Hohmann, S., and Gordon, E. (2008). Production, characterization and crystallization of the *Plasmodium falciparum* aquaporin. *Protein Expr Purif* 59, 69-78.
- Herrick, J., and Sclavi, B. (2007). Ribonucleotide reductase and the regulation of DNA replication: An old story and an ancient heritage. *Mol Microbiol* 63, 22-34.
- Hillier, C.J., Ware, L.A., Barbosa, A., Angov, E., Lyon, J.A., Heppner, D.G., and Lanar, D.E. (2005). Process development and analysis of liver-stage antigen 1, a preerythrocyte-stage protein-based vaccine for *Plasmodium falciparum*. *Infect Immun* 73, 2109-2115.
- Ho, S.P., Britton, D.H., Stone, B.A., Behrens, D.L., Leffert, L.M., Hobbs, F.W., Miller, J.A., and Trainor, G.L. (1996). Potent antisense oligonucleotides to the human multidrug resistance-1 mRNA are rationally selected by mapping RNA-accessible sites with oligonucleotide libraries. *Nucleic Acids Res* 24, 1901-1907.
- Hodges, Y.K., Antholine, W.E., and Horwitz, L.D. (2004). Effect on ribonucleotide reductase of novel lipophilic iron chelators: The desferri-exochelins. *Biochem Biophys Res Comm* 315, 595-598.
- Hofer, A., Schmidt, P.P., Gräslund, A., and Thelander, L. (1997). Cloning and characterization of the R1 and R2 subunits of ribonucleotide reductase from *Trypanosoma brucei*. *Proc Natl Acad Sci Unit States Am* 94, 6959-6964.
- Högbom, M. (2010). The manganese/iron-carboxylate proteins: What is what, where are they, and what can the sequences tell us? *J Biol Inorg Chem* 15, 339-349.
- Högbom, M., Stenmark, P., Voevodskaya, N., McClarty, G., Gräslund, A., and Nordlund, P. (2004). The radical site in chlamydial ribonucleotide reductase defines a new R2 subclass. *Science* 305, 245-248.
- Holland, K.P., Elford, H.L., Bracchi-Ricard, V., Annis, C.G., Schuster, S.M., and Chakrabarti, D. (1998). Antimalarial activities of polyhydroxyphenyl and hydroxamic acid derivatives. *Antimicrob Agents Chemother* 42, 2456-2458.
- Huang, M., and Elledge, S.J. (1997). Identification of RNR4, encoding a second essential small subunit of ribonucleotide reductase in *Saccharomyces cerevisiae*. *Mol Cell Biol* 17, 6105-6113.
- Huthmacher, C., Hoppe, A., Bulik, S., and Holzhütter, H.-G. (2010). Antimalarial drug targets in *Plasmodium falciparum* predicted by stage-specific metabolic network analysis. *BMC Syst Biol* 4, 120.
- Ingemarson, R., and Thelander, L. (1996). A kinetic study on the influence of nucleoside triphosphate effectors on subunit interaction in mouse ribonucleotide reductase. *Biochemistry* 35, 8603-8609.
- Ingram, G.M., and Kinnaird, J.H. (1999). Ribonucleotide reductase: A new target for antiparasite therapies. *Parasitol Today* 15, 338-342.
- Inselburg, J., and Banyal, H.S. (1984). Synthesis of DNA during the asexual cycle of *Plasmodium falciparum* in culture. *Mol Biochem Parasitol* 10, 79-87.
- Janoušková, J., Horák, A., Oborník, M., Lukeš, J., and Keeling, P.J. (2010). A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. *Proc Natl Acad Sci Unit States Am* 107, 10949-10954.
- Jeha, S., Gandhi, V., Chan, K.W., McDonald, L., Ramirez, I., Madden, R., Rytting, M., Brandt, M., Keating, M., Plunkett, W., et al. (2004). Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia. *Blood* 103, 784-789.
- Jordan, A., Pontis, E., Atta, M., Krook, M., Gibert, I., Barbé, J., and Reichard, P. (1994). A second class I ribonucleotide reductase in Enterobacteriaceae: Characterization of the *Salmonella typhimurium* enzyme. *Proc Natl Acad Sci Unit States Am* 91, 12892-12896.
- Jordan, A., and Reichard, P. (1998). Ribonucleotide reductases. *Annu Rev Biochem* 67, 71-98.
- Khan, A.U. (2006). Ribozyme: a clinical tool. *Clin Chim Acta* 367, 20-27.
- Kolberg, M., Strand, K.R., Graff, P., and Andersson, K.K. (2004). Structure, function, and mechanism of ribonucleotide reductases. *Biochim Biophys Acta* 1699, 1-34.
- Krakoff, I.H., Brown, N.C., and Reichard, P. (1968). Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Canc Res* 28, 1559-1565.
- Krug, E.C., Marr, J.J., and Berens, R.L. (1989). Purine metabolism in *Toxoplasma gondii*. *J Biol Chem* 264, 10601-10607.
- Kumar, D., Viberg, J., Nilsson, A.K., and Chabes, A. (2010). Highly mutagenic and severely imbalanced dNTP pools can escape detection by the S-phase checkpoint. *Nucleic Acids Res*.
- Kumar, R., Adams, B., Oldenburg, A., Musiyenko, A., and Barik, S. (2002). Characterisation and expression of a PP1 serine/threonine protein phosphatase (PfPPP1) from the malaria parasite, *Plasmodium falciparum*: Demonstration of its essential role using RNA interference. *Malaria Journal* 1, 5.
- Kyes, S., Christodoulou, Z., Pinches, R., and Newbold, C. (2002). Stage-specific merozoite surface protein 2 antisense transcripts in *Plasmodium falciparum*. *Mol Biochem Parasitol* 123, 79-83.
- Lane, J., Martin, T.A., and Jiang, W.G. (2010). Targeting RhoC by way of ribozyme transgene in human breast cancer cells and its impact on cancer invasion. *World J Oncol* 1, 7.
- Leander, B.S., and Keeling, P.J. (2003). Morphostasis in alveolate evolution. *Trends Ecol Evol* 18, 395-402.
- Lee, S.H., and Sinko, P.J. (2006). siRNA—Getting the message out. *Eur J Pharm Sci* 27, 401-410.
- Lee, Y., Vassilakos, A., Feng, N., Lam, V., Xie, H., Wang, M., Jin, H., Xiong, K., Liu, C., Wright, J., et al. (2003). GTI-2040, an antisense agent targeting the small subunit component (R2) of human ribonucleotide reductase, shows potent antitumor activity against a variety of tumors. *Canc Res* 63, 2802-2811.
- Lepoivre, M., Fieschi, F., Coves, J., Thelander, L., and Fontecave, M. (1991). Inactivation of ribonucleotide reductase by nitric oxide. *Biochem Biophys Res Comm* 179, 442-448.

- Levine, N.D. (1988). Progress in taxonomy of the apicomplexan protozoa. *J Protozool* 35, 518-520.
- Lewin, A.S., and Hauswirth, W.W. (2001). Ribozyme gene therapy: Applications for molecular medicine. *Trends Mol Med* 7, 221-228.
- Li, N.-S., Frederiksen, J.K., Koo, S.C., Lu, J., Wilson, T.J., Lilley, D.M.J., and Piccirilli, J.A. (2010). A general and efficient approach for the construction of RNA oligonucleotides containing a 5'-phosphorothiolate linkage. *Nucleic Acids Res*, 1-16.
- Lin, A.L., and Elford, H.L. (1980). Adenosine deaminase impairment and ribonucleotide reductase activity and levels in HeLa cells. *J Biol Chem* 255, 8523-8528.
- Lin, Z.P., Belcourt, M.F., Cory, J.G., and Sartorelli, A.C. (2004). Stable suppression of the R2 subunit of ribonucleotide reductase by R2-targeted short interference RNA sensitizes p53(-/-) HCT-116 colon cancer cells to DNA-damaging agents and ribonucleotide reductase inhibitors. *J Biol Chem* 279, 27030-27038.
- Liuzzi, M., Déziel, R., Moss, N., Beaulieu, P., Bonneau, A.M., Bousquet, C., Chafouleas, J.G., Garneau, M., Jaramillo, J., and Krogsrud, R.L. (1994). A potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity *in vivo*. *Nature* 372, 695-698.
- Lizundia, R., Werling, D., Langsley, G., and Ralph, S.A. (2009). *Theileria* apicoplast as a target for chemotherapy. *Antimicrob Agents Chemother* 53, 1213-1217.
- Llinás, M., Bozdech, Z., Wong, E.D., Adai, A.T., and DeRisi, J.L. (2006). Comparative whole genome transcriptome analysis of three *Plasmodium falciparum* strains. *Nucleic Acids Res* 34, 1166-1173.
- Logan, D.T. (2011). Closing the circle on ribonucleotide reductases. *Nat Struct Biol* 18, 251-253.
- Lou, Z., and Zhang, X. (2010). Protein targets for structure-based anti-*Mycobacterium tuberculosis* drug discovery. *Protein Cell* 1, 435-442.
- Lu, Y., Gu, J., Jin, D., Gao, Y., and Yuan, M. (2011). Inhibition of telomerase activity by HDV ribozyme in cancers. *J Exp Clin Oncol* 30, 1.
- Lundin, D., Torrents, E., Poole, A.M., and Sjöberg, B.-M. (2009). RNRdb, a curated database of the universal enzyme family ribonucleotide reductase, reveals a high level of misannotation in sequences deposited to GenBank. *BMC Genomics* 10, 589.
- Lytton, S.D., Mester, B., Libman, J., Shanzer, A., and Cabantchik, Z.I. (1994). Mode of action of iron (III) chelators as antimalarials: II. Evidence for differential effects on parasite iron-dependent nucleic acid synthesis. *Blood* 84, 910-915.
- Madrid, D.C., Ting, L.-M., Waller, K.L., Schramm, V.L., and Kim, K. (2008). *Plasmodium falciparum* purine nucleoside phosphorylase is critical for viability of malaria parasites. *J Biol Chem* 283, 35899-35907.
- Malhotra, P., Dasaradhi, P.V.N., Kumar, A., Mohammed, A., Agrawal, N., Bhatnagar, R.K., and Chauhan, V.S. (2002). Double-stranded RNA-mediated gene silencing of cysteine proteases (falcipain-1 and -2) of *Plasmodium falciparum*. *Mol Microbiol* 45, 1245-1254.
- Mathews, C.K. (2006). DNA precursor metabolism and genomic stability. *FASEB J* 20, 1300-1314.
- McFadden, G.I., and Roos, D.S. (1999). Apicomplexan plastids as drug targets. *Trends Microbiol* 7, 328-333.
- McRobert, L., and McConkey, G.A. (2002). RNA interference (RNAi) inhibits growth of *Plasmodium falciparum*. *Mol Biochem Parasitol* 119, 273-278.
- Mdluli, K., and Spigelman, M. (2006). Novel targets for tuberculosis drug discovery. *Curr Opin Pharmacol* 6, 459-467.
- Militello, K.T., Patel, V., Chessler, A.-D., Fisher, J.K., Kasper, J.M., Gunasekera, A.M., and Wirth, D.F. (2005). RNA polymerase II synthesizes antisense RNA in *Plasmodium falciparum*. *RNA* 11, 365-370.
- Ming, X., Alam, M.R., Fisher, M., Yan, Y., Chen, X., and Juliano, R.L. (2010). Intracellular delivery of an antisense oligonucleotide via endocytosis of a G protein-coupled receptor. *Nucleic Acids Res*.
- Mkulama, M.A.P., Chishimba, S., Sikalima, J., Rouse, P., Thuma, P.E., and Mharakurwa, S. (2008). Escalating *Plasmodium falciparum* antifolate drug resistance mutations in Macha, rural Zambia. *Malaria Journal* 7, 87.
- Mohammed, A., Dasaradhi, P.V.N., Bhatnagar, R.K., Chauhan, V.S., and Malhotra, P. (2003). *In vivo* gene silencing in *Plasmodium berghei*—a mouse malaria model. *Biochem Biophys Res Comm* 309, 506-511.
- Morrison, D.A. (2009). Evolution of the Apicomplexa: Where are we now? *Trends Parasitol* 25, 375-382.
- Moss, N., Déziel, R., Adams, J., Aubry, N., Bailey, M., Baillet, M., Beaulieu, P., DiMaio, J., Duceppe, J.S., Ferland, J.M., *et al.* (1993). Inhibition of herpes simplex virus type 1 ribonucleotide reductase by substituted tetrapeptide derivatives. *J Med Chem* 36, 3005-3009.
- Mu, J., Seydel, K.B., Bates, A., and Su, X.-z. (2010). Recent progress in functional genomic research in *Plasmodium falciparum*. *Curr Genom* 11, 279-286.
- Nayak, A., and Kohli, D. (2005). Ribozymes: The trans acting tools for RNA manipulation. *Indian Journal of Pharmaceutical Education* 39, 92-96.
- Nguyen, H.H., Ge, J., Perlstein, D.L., and Stubbe, J. (1999). Purification of ribonucleotide reductase subunits Y1, Y2, Y3, and Y4 from yeast: Y4 plays a key role in diiron cluster assembly. *Proc Natl Acad Sci Unit States Am* 96, 12339-12344.
- Nivez, M., Achbarou, A., Bienvenu, J.D., Mazier, D., Doerig, C., and Vaquero, C. (2000). A study of selected *Plasmodium yoelii* messenger RNAs during hepatocyte infection. *Mol Biochem Parasitol* 111, 31-39.
- Nocentini, G. (1996). Ribonucleotide reductase inhibitors: New strategies for cancer chemotherapy. *Crit Rev Oncol Hematol* 22, 89-126.
- Nocentini, G., Federici, F., Armellini, R., Franchetti, P., and Barzi, A. (1990). Isolation of two cellular lines resistant to ribonucleotide reductase inhibitors to investigate the inhibitory activity of 2,2'-bipyridyl-6-carbothioamide. *Anti Canc Drugs* 1, 171-177.
- Noonpakdee, W., Pothikasikorn, J., Nimitsantiwong, W., and Wilairat, P. (2003). Inhibition of *Plasmodium falciparum* proliferation *in vitro* by antisense oligodeoxynucleotides against malarial topoisomerase II. *Biochem Biophys Res Comm* 302, 659-664.
- Nordlund, P., and Eklund, H. (1993). Structure and function of the *Escherichia coli* ribonucleotide reductase protein R2. *J Mol Biol* 232, 123-164.
- Nordlund, P., and Reichard, P. (2006). Ribonucleotide reductases. *Annu Rev Biochem* 75, 681-706.

- Nordlund, P., Sjöberg, B.-M., and Eklund, H. (1990). Three-dimensional structure of the free radical protein of ribonucleotide reductase. *Nature* **345**, 593-598.
- Norris, J.S., Hoel, B., Voeks, D., Maggouta, F., Dahm, M., Pan, W., and Clawson, G. (2000). Design and testing of ribozymes for cancer gene therapy. *Adv Exp Med Biol* **465**, 293-301.
- Nosrati, M., Li, S., Bagheri, S., Ginzinger, D., Blackburn, E.H., Debs, R.J., and Kashani-Sabet, M. (2004). Antitumor activity of systemically delivered ribozymes targeting murine telomerase RNA. *Clin Cancer Res* **10**, 4983-4990.
- Nyholm, S., Mann, G.J., Johansson, A.G., Bergeron, R.J., Gräslund, A., and Thelander, L. (1993). Role of ribonucleotide reductase in inhibition of mammalian cell growth by potent iron chelators. *J Biol Chem* **268**, 26200-26205.
- Olliaro, P.L., and Yuthavong, Y. (1999). An overview of chemotherapeutic targets for antimalarial drug discovery. *Pharmacol Therapeut* **81**, 91-110.
- Ortigosa, A.D., Hristova, D., Perlstein, D.L., Zhang, Z., Huang, M., and Stubbe, J. (2006). Determination of the *in vivo* stoichiometry of tyrosyl radical per $\beta\beta'$ in *Saccharomyces cerevisiae* ribonucleotide reductase. *Biochemistry* **45**, 12282-12294.
- Padmanaban, G. (2003). Drug targets in malaria parasites. *Adv Biochem Eng Biotechnol* **84**, 123-141.
- Paradis, H., Gaudreau, P., Brazeau, P., and Langelier, Y. (1988). Mechanism of inhibition of herpes simplex virus (HSV) ribonucleotide reductase by a nonapeptide corresponding to the carboxyl terminus of its subunit 2. Specific binding of a photoaffinity analog, [4'-azido-Phe6] HSV H2-6(6-15), to subunit 1. *J Biol Chem* **263**, 16045-16050.
- Parker, M.D., Hyde, R.J., Yao, S.Y., McRobert, L., Cass, C.E., Young, J.D., McConkey, G.A., and Baldwin, S.A. (2000). Identification of a nucleoside/nucleobase transporter from *Plasmodium falciparum*, a novel target for anti-malarial chemotherapy. *Biochem J* **349**, 67-75.
- Patankar, S., Munasinghe, A., Shoaibi, A., Cummings, L.M., and Wirth, D.F. (2001). Serial analysis of gene expression in *Plasmodium falciparum* reveals the global expression profile of erythrocytic stages and the presence of anti-sense transcripts in the malarial parasite. *Mol Biol Cell* **12**, 3114-3125.
- Pellegrini, M., Liehr, S., Fisher, A.L., Laub, P.B., Cooperman, B.S., and Mierke, D.F. (2000). Structure-based optimization of peptide inhibitors of mammalian ribonucleotide reductase. *Biochemistry* **39**, 12210-12215.
- Pender, B.A., Wu, X., Axelsen, P.H., and Cooperman, B.S. (2001). Toward a rational design of peptide inhibitors of ribonucleotide reductase: Structure—function and modeling studies. *J Med Chem* **44**, 36-46.
- Pereira, S., Cerqueira, N.M.F.S.A., Fernandes, P.A., and Ramos, M.J. (2006). Computational studies on class I ribonucleotide reductase: Understanding the mechanisms of action and inhibition of a cornerstone enzyme for the treatment of cancer. *Eur Biophys J* **35**, 125-135.
- Pereira, S., Fernandes, P.A., and Ramos, M.J. (2004). Mechanism for ribonucleotide reductase inactivation by the anticancer drug gemcitabine. *J Comput Chem* **25**, 1286-1294.
- Perez, M., Cerqueira, N., Fernandes, P., and Ramos, M. (2010). Ribonucleotide reductase: A mechanistic portrait of substrate analogues inhibitors. *Curr Med Chem* **17**, 2854-2872.
- Perlstein, D.L., Ge, J., Ortigosa, A.D., Robblee, J.H., Zhang, Z., Huang, M., and Stubbe, J. (2005). The active form of the *Saccharomyces cerevisiae* ribonucleotide reductase small subunit is a heterodimer *in vitro* and *in vivo*. *Biochemistry* **44**, 15366-15377.
- Pouvelle, B., Spiegel, R., Hsiao, L., Howard, R.J., Morris, R.L., Thomas, A.P., and Taraschi, T.F. (1991). Direct access to serum macromolecules by intraerythrocytic malaria parasites. *Nature* **353**, 73-75.
- Pradines, B., Ramiandrasoa, F., Basco, L.K., Bricard, L., Kunesch, G., and Le Bras, J. (1996). *In vitro* activities of novel catecholate siderophores against *Plasmodium falciparum*. *Antimicrob Agents Chemother* **40**, 2094-2098.
- Prusty, D., Dar, A., Priya, R., Sharma, A., Dana, S., Choudhury, N.R., Rao, N.S., and Dhar, S.K. (2010). Single-stranded DNA binding protein from human malarial parasite *Plasmodium falciparum* is encoded in the nucleus and targeted to the apicoplast. *Nucleic Acids Res*.
- Puerta-Fernández, E., Romero-López, C., Barroso-delJesus, A., and Berzal-Herranz, A. (2003). Ribozymes: Recent advances in the development of RNA tools. *FEMS Microbiol Rev* **27**, 75-97.
- Ralph, S., van Dooren, G., Waller, R., Crawford, M.J., Fraunholz, M., Foth, B.J., Tonkin, C., Roos, D., and McFadden, G.I. (2004). Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat Rev Microbiol* **2**, 203-216.
- Ralph, S.A., D'Ombrain, M.C., and McFadden, G.I. (2001). The apicoplast as an antimalarial drug target. *Drug Resist Updates* **4**, 145-151.
- Raman, J., and Balaram, H. (2004). Nucleotide metabolism in *Plasmodium falciparum*: Recent developments. *Med Chem Rev Online* **1**, 465-473.
- Rapaport, E., Misiura, K., Agrawal, S., and Zamecnik, P. (1992). Antimalarial activities of oligodeoxynucleotide phosphorothioates in chloroquine-resistant *Plasmodium falciparum*. *Proc Natl Acad Sci Unit States Am* **89**, 8577-8580.
- Reichard, P. (1988). Interactions between deoxyribonucleotide and DNA synthesis. *Annu Rev Biochem* **57**, 349-374.
- Reichard, P. (1997). The evolution of ribonucleotide reduction. *Trends Biochem Sci* **22**, 81-85.
- Reichard, P. (2002). Ribonucleotide reductases: The evolution of allosteric regulation. *Arch Biochem Biophys* **397**, 149-155.
- Reichard, P. (2010). Ribonucleotide reductases: Substrate specificity by allostery. *Biochem Biophys Res Comm* **396**, 19-23.
- Reichard, P., Baldesten, A., and Rutberg, L. (1961). Formation of deoxycytidine phosphates from cytidine phosphates in extracts from *Escherichia coli*. *J Biol Chem* **236**, 1150.
- Reichard, P., Eliasson, R., Ingemarson, R., and Thelander, L. (2000). Cross-talk between the allosteric effector-binding sites in mouse ribonucleotide reductase. *J Biol Chem* **275**, 33021-33026.

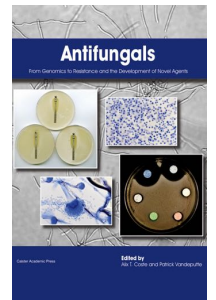
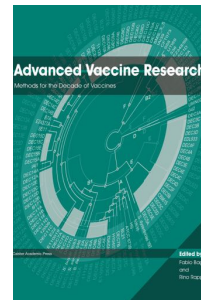
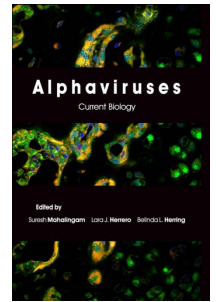
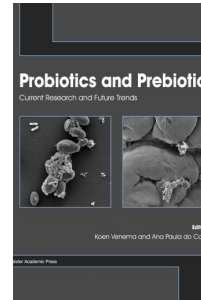
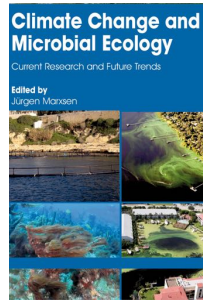
- Richardson, D.R. (2002). Iron chelators as therapeutic agents for the treatment of cancer. *Crit Rev Oncol Hematol* 42, 267-281.
- Robins, M.J. (1999). Mechanism-based inhibition of ribonucleotide reductases: New mechanistic considerations and promising biological applications. *Nucleos Nucleot Nucleic Acids* 18, 779-793.
- Rofougaran, R., Vodnala, M., and Hofer, A. (2006). Enzymatically active mammalian ribonucleotide reductase exists primarily as an $\alpha_6\beta_2$ octamer. *J Biol Chem* 281, 27705-27711.
- Roos, D.S., Crawford, M.J., Donald, R.G.K., Fraunholz, M., Harb, O.S., He, C.Y., Kissinger, J.C., Shaw, M.K., and Striepen, B. (2002). Mining the *Plasmodium* genome database to define organellar function: What does the apicoplast do? *Phil Trans Roy Soc Lond B Biol Sci* 357, 35-46.
- Roos, D.S., Crawford, M.J., Donald, R.G.K., Kissinger, J.C., Klimczak, L.J., and Striepen, B. (1999). Origin, targeting, and function of the apicomplexan plastid. *Curr Opin Microbiol* 2, 426-432.
- Roshick, C., Iliffe-Lee, E.R., and McClarty, G. (2000). Cloning and characterization of ribonucleotide reductase from *Chlamydia trachomatis*. *J Biol Chem* 275, 38111-38119.
- Rougemont, M., Van Saanen, M., Sahli, R., Hinrikson, H.P., Bille, J., and Jaton, K. (2004). Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol* 42, 5636-5643.
- Rova, U., Adrait, A., Pötsch, S., Gräslund, A., and Thelander, L. (1999). Evidence by mutagenesis that Tyr³⁷⁰ of the mouse ribonucleotide reductase R2 protein is the connecting link in the intersubunit radical transfer pathway. *J Biol Chem* 274, 23746-23751.
- Rowe, A.K., Rowe, S.Y., Snow, R.W., Korenromp, E.L., Schellenberg, J.R.A., Stein, C., Nahlen, B.L., Bryce, J., Black, R.E., and Steketee, R.W. (2006). The burden of malaria mortality among African children in the year 2000. *Int J Epidemiol* 35, 691-704.
- Rubin, H., Salem, J.S., Li, L.S., Yang, F.D., Mama, S., Wang, Z.M., Fisher, A., Hamann, C.S., and Cooperman, B.S. (1993). Cloning, sequence determination, and regulation of the ribonucleotide reductase subunits from *Plasmodium falciparum*: A target for antimalarial therapy. *Proc Natl Acad Sci Unit States Am* 90, 9280-9284.
- Sachs, J., and Malaney, P. (2002). The economic and social burden of malaria. *Nature* 415, 680-685.
- Saei, A.A., and Ahmadian, S. (2009). Ribonucleotide reductase inhibitors with erythropoietin and iron sulfate against malaria. *Medical hypotheses* 72, 611.
- Sahu, N.K., Shilakari, G., Nayak, A., and Kohli, D.V. (2007). Antisense technology: A selective tool for gene expression regulation and gene targeting. *Curr Pharmaceut Biotechnol* 8, 291-304.
- Salowe, S., Bollinger, J.M., Ator, M., Stubbe, J., McCracken, J., Peisach, J., Samano, M.C., and Robins, M.J. (1993). Alternative model for mechanism-based inhibition of *Escherichia coli* ribonucleotide reductase by 2'-azido-2'-deoxyuridine 5'-diphosphate. *Biochemistry* 32, 12749-12760.
- Santini, D., Tonini, G., Abbate, A., Di Cosimo, S., Gravante, G., Vincenzi, B., Campisi, C., Patti, G., and Di Sciascio, G. (2000). Gemcitabine-induced atrial fibrillation: A hitherto unreported manifestation of drug toxicity. *Ann Oncol* 11, 479-481.
- Sato, S., and Wilson, R.J.M. (2005). The plastid of *Plasmodium* spp.: A target for inhibitors. *Curr Top Microbiol Immunol* 295, 251-273.
- Scherr, M., Steinmann, D., and Eder, M. (2004). RNA interference (RNAi) in hematology. *Annals of Hematology* 83, 1-8.
- Schneider, T.D., and Stephens, R.M. (1990). Sequence logos: a new way to display consensus sequences. *Nucleic Acids Res* 18, 6097-6100.
- Shao, J., Zhou, B., Chu, B., and Yen, Y. (2006). Ribonucleotide reductase inhibitors and future drug design. *Current Cancer Drug Targets* 6, 409-431.
- Singh, B., Kim Sung, L., Matusop, A., Radhakrishnan, A., Shamsul, S.S.G., Cox-Singh, J., Thomas, A., and Conway, D.J. (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 363, 1017-1024.
- Sjöberg, B.-M. (1997). Ribonucleotide reductases □ A group of enzymes with different metallosites and a similar reaction mechanism. *Struct Bond* 88, 139-173.
- Sjöberg, B.-M. (2010). A Never-Ending Story. *Science* 329, 1475-1476.
- Smith, B.D., and Karp, J.E. (2003). Ribonucleotide reductase: An old target with new potential. *Leuk Res* 27, 1075-1076.
- Sneeden, J.L., and Loeb, L.A. (2004). Mutations in the R2 subunit of ribonucleotide reductase that confer resistance to hydroxyurea. *J Biol Chem* 279, 40723-40728.
- Spielman, D.J. (2009). XVI. Public-private partnerships and pro-poor livestock research: The search for an East Coast fever vaccine. Enhancing the Effectiveness of Sustainability Partnerships: Summary of a Workshop *National Research Council of the National Academies. Washinton D.C.: The National Academies Press.* 122 p.
- Sridaran, S., McClintock, S.K., Syphard, L.M., Herman, K.M., Barnwell, J.W., and Udhayakumar, V. (2010). Anti-folate drug resistance in Africa: Meta-analysis of reported dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) mutant genotype frequencies in African *Plasmodium falciparum* parasite populations. *Malaria Journal* 9, 247.
- Sriwilaijaroen, N., Boonma, S., Attasart, P., Pothikasikorn, J., Panyim, S., and Noonpakdee, W. (2009). Inhibition of *Plasmodium falciparum* proliferation *in vitro* by double-stranded RNA directed against malaria histone deacetylase. *Biochem Biophys Res Comm* 381, 144-147.
- Striepen, B., Pruijssers, A.J.P., Huang, J., Li, C., Gubbels, M.-J., Umejiego, N.N., Hedstrom, L., and Kissinger, J.C. (2004). Gene transfer in the evolution of parasite nucleotide biosynthesis. *Proc Natl Acad Sci Unit States Am* 101, 3154-3159.
- Stubbe, J., Nocera, D.G., Yee, C.S., and Chang, M.C.Y. (2003). Radical initiation in the class I ribonucleotide reductase: Long-range proton-coupled electron transfer? *Chem Rev* 103, 2167-2201.

- Sunil, S., Hossain, M.J., Ramasamy, G., and Malhotra, P. (2008). Transient silencing of *Plasmodium falciparum* Tudor Staphylococcal Nuclease suggests an essential role for the protein. *Biochem Biophys Res Comm* 372, 373-378.
- Szekeres, T., Fritzer-Szekeres, M., and Elford, H.L. (1997). The enzyme ribonucleotide reductase: Target for antitumor and anti-HIV therapy. *Crit Rev Clin Lab Sci* 34, 503-528.
- Takala, S.L., and Plowe, C.V. (2009). Genetic diversity and malaria vaccine design, testing and efficacy: Preventing and overcoming 'vaccine resistant malaria'. *Parasite Immunol* 31, 560-573.
- Tanaka, H., Arakawa, H., Yamaguchi, T., Shiraishi, K., Fukuda, S., Matsui, K., Takei, Y., and Nakamura, Y. (2000). A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* 404, 42-49.
- Tedeschi, L., Lande, C., Cecchetti, A., and Citti, L. (2009). Hammerhead ribozymes in therapeutic target discovery and validation. *Drug Discov Today* 14, 776-783.
- Thelander, L., and Reichard, P. (1979). Reduction of ribonucleotides. *Annu Rev Biochem* 48, 133-158.
- Ting, L.-M., Shi, W., Lewandowicz, A., Singh, V., Mwakingwe, A., Birck, M.R., Ringia, E.A.T., Bench, G., Madrid, D.C., Tyler, P.C., et al. (2005). Targeting a novel *Plasmodium falciparum* purine recycling pathway with specific immucillins. *J Biol Chem* 280, 9547-9554.
- Torrents, E., and Sjöberg, B.-M. (2010). Antibacterial activity of radical scavengers against class Ib ribonucleotide reductase from *Bacillus anthracis*. *Biol Chem* 391, 229-234.
- Tracy, S.M., and Sherman, I.W. (1972). Purine uptake and utilization by the avian malaria parasite *Plasmodium lophurae*. *J Protozool* 19, 541-549.
- Turner, P.C. (2000). Ribozymes. Their design and use in cancer. *Adv Exp Med Biol* 465, 303-318.
- Tuteja, R., Pradhan, A., and Sharma, S. (2008). *Plasmodium falciparum* signal peptidase is regulated by phosphorylation and required for intra-erythrocytic growth. *Mol Biochem Parasitol* 157, 137-147.
- Ullu, E., Tschudi, C., and Chakraborty, T. (2004). RNA interference in protozoan parasites. *Cell Microbiol* 6, 509-519.
- Uppsten, M., Färnegårdh, M., Domkin, V., and Uhlin, U. (2006). The first holocomplex structure of ribonucleotide reductase gives new insight into its mechanism of action. *J Mol Biol* 359, 365-377.
- Vaish, N., Chen, F., Seth, S., Fosnaugh, K., Liu, Y., Adami, R., Brown, T., Chen, Y., Harvie, P., Johns, R., et al. (2010). Improved specificity of gene silencing by siRNAs containing unlocked nucleobase analogs. *Nucleic Acids Res*, 1-10.
- Vedadi, M., Lew, J., Artz, J., Amani, M., Zhao, Y., Dong, A., Wasney, G.A., Gao, M., Hills, T., Brokx, S., et al. (2007). Genome-scale protein expression and structural biology of *Plasmodium falciparum* and related Apicomplexan organisms. *Mol Biochem Parasitol* 151, 100-110.
- Voegtli, W.C., Ge, J., Perlestein, D.L., Stubbe, J., and Rosenzweig, A.C. (2001). Structure of the yeast ribonucleotide reductase Y2Y4 heterodimer. *Proc Natl Acad Sci Unit States Am* 98, 10073-10078.
- Waller, R.F., and McFadden, G.I. (2005). The apicoplast: A review of the derived plastid of apicomplexan parasites. *Curr Issues Mol Biol* 7, 57-79.
- Wang, P.J., Chabes, A., Casagrande, R., Tian, X.C., Thelander, L., and Huffaker, T.C. (1997). Rnr4p, a novel ribonucleotide reductase small-subunit protein. *Mol Cell Biol* 17, 6114-6121.
- Weber, J.L. (1987). Analysis of sequences from the extremely A + T-rich genome of *Plasmodium falciparum*. *Gene* 52, 103-109.
- Wheeler, L.J., Rajagopal, I., and Mathews, C.K. (2005). Stimulation of mutagenesis by proportional deoxyribonucleoside triphosphate accumulation in *Escherichia coli*. *DNA Repair* 4, 1450-1456.
- White, N.J. (2004). Antimalarial drug resistance. *J Clin Invest* 113, 1084-1092.
- Whitnall, M., Howard, J., Ponka, P., and Richardson, D.R. (2006). A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proc Natl Acad Sci Unit States Am* 103, 14901-14906.
- Wiesner, J., Reichenberg, A., Heinrich, S., Schlitzer, M., and Jomaa, H. (2008). The plastid-like organelle of apicomplexan parasites as drug target. *Curr Pharmaceut Des* 14, 855-871.
- Winzeler, E.A. (2008). Malaria research in the post-genomic era. *Nature* 455, 751-756.
- Xue, X., Zhang, Q., Huang, Y., Feng, L., and Pan, W. (2008). No miRNA were found in *Plasmodium* and the ones identified in erythrocytes could not be correlated with infection. *Malaria Journal* 7, 47.
- Yanamoto, S., Iwamoto, T., Kawasaki, G., Yoshitomi, I., Baba, N., and Mizuno, A. (2005). Silencing of the p53R2 gene by RNA interference inhibits growth and enhances 5-fluorouracil sensitivity of oral cancer cells. *Canc Lett* 223, 67-76.
- Yang, F., Curran, S.C., Li, L.S., Avarbock, D., Graf, J.D., Chua, M.M., Lu, G., Salem, J., and Rubin, H. (1997). Characterization of two genes encoding the *Mycobacterium tuberculosis* ribonucleotide reductase small subunit. *J Bacteriol* 179, 6408-6415.
- Yang, F., Spanevello, R., Celiker, I., Hirschmann, R., Rubin, H., and Cooperman, B.S. (1990). The carboxyl terminus heptapeptide of the R2 subunit of mammalian ribonucleotide reductase inhibits enzyme activity and can be used to purify the R1 subunit. *FEBS Lett* 272, 61-64.
- Yeh, I., and Altman, R.B. (2006). Drug targets for *Plasmodium falciparum*: A post-genomic review/survey. *Mini-Rev Med Chem* 6, 177-202.
- Yeh, I., Hanekamp, T., Tsoka, S., Karp, P.D., and Altman, R.B. (2004). Computational analysis of *Plasmodium falciparum* metabolism: Organizing genomic information to facilitate drug discovery. *Genome Res* 14, 917-924.
- Yen, Y. (2003). Ribonucleotide reductase subunit one as gene therapy target: commentary re: M-Y. Cao et al., Adenovirus-mediated ribonucleotide reductase R1 gene therapy of human colon adenocarcinoma. *Clin. Cancer Res.*, 9: 4304-4308, 2003. *Clin Cancer Res* 9, 4304-4308.

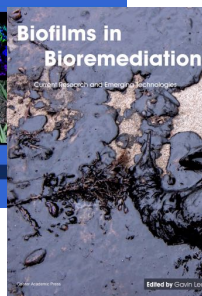
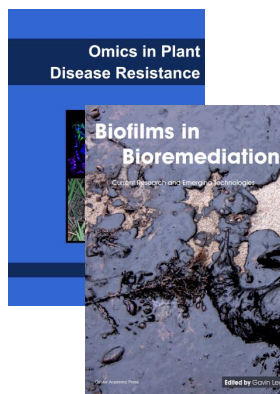
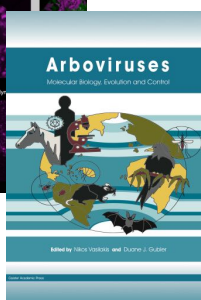
Further Reading

Caister Academic Press is a leading academic publisher of advanced texts in microbiology, molecular biology and medical research. Full details of all our publications at [caister.com](http://www.caister.com)

- **MALDI-TOF Mass Spectrometry in Microbiology**
Edited by: M Kostrzewa, S Schubert (2016)
www.caister.com/malditof
- **Aspergillus and Penicillium in the Post-genomic Era**
Edited by: RP Vries, IB Gelber, MR Andersen (2016)
www.caister.com/aspergillus2
- **The Bacteriocins: Current Knowledge and Future Prospects**
Edited by: RL Dorit, SM Roy, MA Riley (2016)
www.caister.com/bacteriocins
- **Omics in Plant Disease Resistance**
Edited by: V Bhaduria (2016)
www.caister.com/opdr
- **Acidophiles: Life in Extremely Acidic Environments**
Edited by: R Quatrini, DB Johnson (2016)
www.caister.com/acidophiles
- **Climate Change and Microbial Ecology: Current Research and Future Trends**
Edited by: J Marxsen (2016)
www.caister.com/climate
- **Biofilms in Bioremediation: Current Research and Emerging Technologies**
Edited by: G Lear (2016)
www.caister.com/biorem
- **Microalgae: Current Research and Applications**
Edited by: MN Tsaloglou (2016)
www.caister.com/microalgae
- **Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives**
Edited by: H Shintani, A Sakudo (2016)
www.caister.com/gasplasma
- **Virus Evolution: Current Research and Future Directions**
Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
www.caister.com/virusevol
- **Arboviruses: Molecular Biology, Evolution and Control**
Edited by: N Vasilakis, DJ Gubler (2016)
www.caister.com/arbo
- **Shigella: Molecular and Cellular Biology**
Edited by: WD Picking, WL Picking (2016)
www.caister.com/shigella
- **Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment**
Edited by: AM Romani, H Guasch, MD Balaguer (2016)
www.caister.com/aquaticbiofilms
- **Alphaviruses: Current Biology**
Edited by: S Mahalingam, L Herrero, B Herring (2016)
www.caister.com/alpha
- **Thermophilic Microorganisms**
Edited by: F Li (2015)
www.caister.com/thermophile



- **Flow Cytometry in Microbiology: Technology and Applications**
Edited by: MG Wilkinson (2015)
www.caister.com/flow
- **Probiotics and Prebiotics: Current Research and Future Trends**
Edited by: K Venema, AP Carmo (2015)
www.caister.com/probiotics
- **Epigenetics: Current Research and Emerging Trends**
Edited by: BP Chadwick (2015)
www.caister.com/epigenetics2015
- **Corynebacterium glutamicum: From Systems Biology to Biotechnological Applications**
Edited by: A Burkovski (2015)
www.caister.com/cory2
- **Advanced Vaccine Research Methods for the Decade of Vaccines**
Edited by: F Bagnoli, R Rappuoli (2015)
www.caister.com/vaccines
- **Antifungals: From Genomics to Resistance and the Development of Novel Agents**
Edited by: AT Coste, P Vandeputte (2015)
www.caister.com/antifungals
- **Bacteria-Plant Interactions: Advanced Research and Future Trends**
Edited by: J Murillo, BA Vinatzer, RW Jackson, et al. (2015)
www.caister.com/bacteria-plant
- **Aeromonas**
Edited by: J Graf (2015)
www.caister.com/aeromonas
- **Antibiotics: Current Innovations and Future Trends**
Edited by: S Sánchez, AL Demain (2015)
www.caister.com/antibiotics
- **Leishmania: Current Biology and Control**
Edited by: S Adak, R Datta (2015)
www.caister.com/leish2
- **Acanthamoeba: Biology and Pathogenesis (2nd edition)**
Author: NA Khan (2015)
www.caister.com/acanthamoeba2
- **Microarrays: Current Technology, Innovations and Applications**
Edited by: Z He (2014)
www.caister.com/microarrays2
- **Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications**
Edited by: D Marco (2014)
www.caister.com/n2



Order from [caister.com/order](http://www.caister.com/order)